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Food Consumption of Mice During Continual Centrifugation

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Abstract. High gravity simulated by continual centrifugation can evoke a slower growth with mice and other organisms. For white mice a transient reduction in food intake also occurs and is believed to be, in part, responsible for the reduced growth. Fields as high as 7 G's were employed. Mice of ages which varied from one to seven weeks at the onset of exposure were studied. A more drastic decrement in food consumption occurred with the older mice at higher field intensities.

Continued exposure to a simulated high gravity can limit the growth of various organisms (Knight, 1806; Matthews; 1953; Gray, 1955; Wunder, et al., 1955, 1959, 1960; Smith, et al., 1959; Walters, et al., 1960; Briney and Wunder, 1960). When of sufficient intensity, this agent can evoke a loss in body mass or even death. As is the case with most physical agents, gravity exerts, primarily, indirect effects upon growth processes. There are a number of intermediate physical and chemical reactions which are finally reflected in the biological response. High gravity causes a given mass of the organism to experience more weight. This, in turn, requires more work to support weight, causes various distortions, and limits the ability of an organism to perform various essential internal and external motions. The primary purpose of this paper is to report that during the first few days of centrifugation the alteration in a mouse's growth pattern is largely attributable to a reduced food intake.

There have been very few basic studies of the relationship between gravitational field intensity and developmental patterns. Until the enthusiasm for space flight started in 1957, there was little interest in the biological effects of prolonged centrifugation. Techniques whereby development can be studied during centrifugation have been developed for only eight forms. These forms are (in addition to fruit fly larvae, mice, and hamster) the bean plant, the wheat seedling, the rat, the chicken, and the turkey. The major technical difficulties are adequate feeding, watering, space for normal movement, and a sufficiently large ratio of centrifuge size to body size. The last would tend to minimize the secondary effects of rotation.

Knight in 1806 demonstrated that centrifugal fields could orient the direction of growth with bean plants. It was not until 1953 that Stephen Gray pursued quantitative studies with such

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fields. He found that intense fields decreased the growth rate of wheat coleoptiles. On the other hand, slower centrifugation actually stimulated plant growth. This enhancement at moderate fields was later confirmed in our laboratory with animals. He also demonstrated that controls supporting lead weights grew slower than experimental coleoptiles supporting comparable weight due to their own mass in an artificially greater gravity. Gray has found that supporting weighted packs did not affect the body size of growing rats. Apparently growth retardation of centrifuged animals can be attributed to something other than a mere increase in effective weight.

The first work with animals appeared in 1953. This consisted of an abstract by Matthews stating that although rats could live quite well at 3 G's, they could not attain normal size. Unfortunately this work has never been described in more detail.

Fruit fly larvae demonstrate a decrease in relative growth rate and in final size as the field increases beyond 1000 G's at 31°C. As would be expected from mechanical considerations, the influence of gravity becomes more pronounced as this animal's size or age increases (Wunder, Herrin, and Cogswell, 1959). The magnitude of this response is drastically altered by temperature (Wunder, Herrin, and Crawford, 1959). Certain evidence exists that these animals adapt in such a manner that they tend to compensate for the extra work imposed upon them by gravitational stress. Upon return to control conditions, the growth constant can exceed the control value and for a given amount of actual growth, less oxygen is consumed (Wunder, Crawford, et al., 1960). Moderate increases in gravity even can stimulate growth.

Successive generations of fowl have been reared at high gravity (Smith et al., 1959). The purpose of this work was to develop and select a super, "high gravity" strain of bipeds. Smith feels that when such a strain is removed from high gravity, a condition comparable to exposing man to less than normal gravity can be mimicked. During centrifugation these animals display a growth pattern similar to that of centrifuged mice. Among the many interesting observations is an increase in relative heart and leg sizes.

After several days or weeks of centrifugation most experimental mice are smaller than the control size (Walters, et al., 1960).

The growth response of mice placed in a new gravitational environment can be roughly broken into three phases: (1) an immediate decrement in growth rate, (2) a period of adaptation

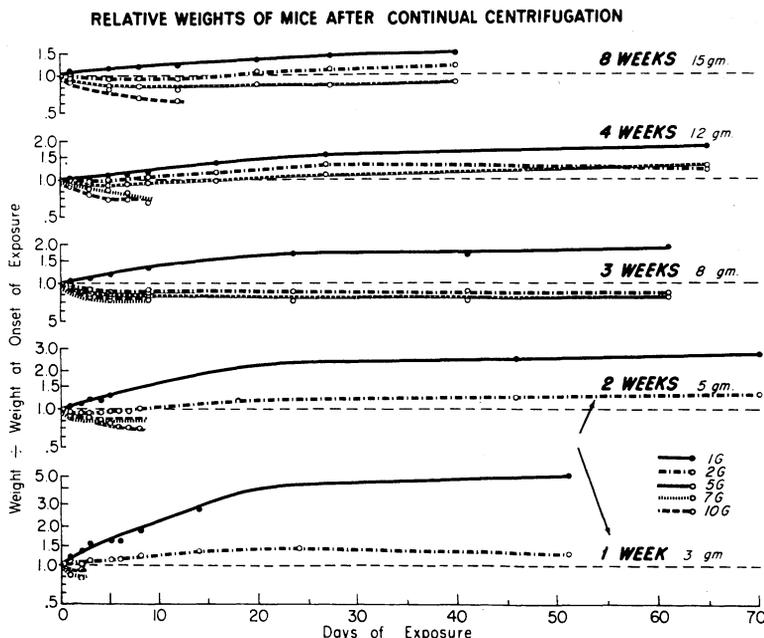RELATIVE WEIGHTS OF MICE AFTER CONTINUAL CENTRIFUGATION

Figure 1. Growth curves for centrifuged mice. Each experimental curve is based upon data for only one group. Mice placed in the centrifuge at two or three weeks of age were nursed by their mothers for a period of several weeks. Age and mass at onset of exposure are indicated.

or adjustment to the new environment, during which time the growth rate recovers toward the control rate, and (3) an eventual, long-term decrement in growth rate which, as a rule, causes the maximum size attained to be somewhat less than the maximum attainable control size.

As with wheat coleoptiles and fly larvae, the initial decrement in relative growth rate for mice becomes greater with higher field intensities. However, contrary to the results with larvae or theoretical expectations, this effect is less marked with the larger or older animals. The phase of initial adaptation or growth recovery is quite discernible. The relative rate of recovery is almost independent of field intensity but is faster for older animals. During this phase, femur growth is accelerated even while the total body mass decreases (Wunder, et al., 1960). The ratio of organ mass to body mass increases for the heart, gastrocnemius muscle, and diaphragm. The onset of the third or long-term effect is faster at the higher fields and with the younger animals. Throughout all phases of this experiment, the experimental animals exhibit less than the normal amount of body fat. Upon removal from the centrifuge, experimental mice eat ravenously and grow at accelerated paces until their

size equals that of the controls. In spite of the adverse environ-
ment, mice have survived and actually grown for a period of
one year at 7 G's. Those at 2 G's have survived for as long as
two years and have actually multiplied.

METHODS AND MATERIALS

After attaining a given age under control conditions, white
mice were placed in a specially designed centrifuge. Experi-
mental animals were kept in the centrifuge throughout the rest
of their life except for periods of approximately ten minutes
each day. Purina "laboratory chow" for mice served as the
major food source. During the first few days of exposure, the
mass of these food pellets were weighed at the beginning and
end of each 24 hour period. Comparable measurements were
performed with uncooked potatoes which served as the only
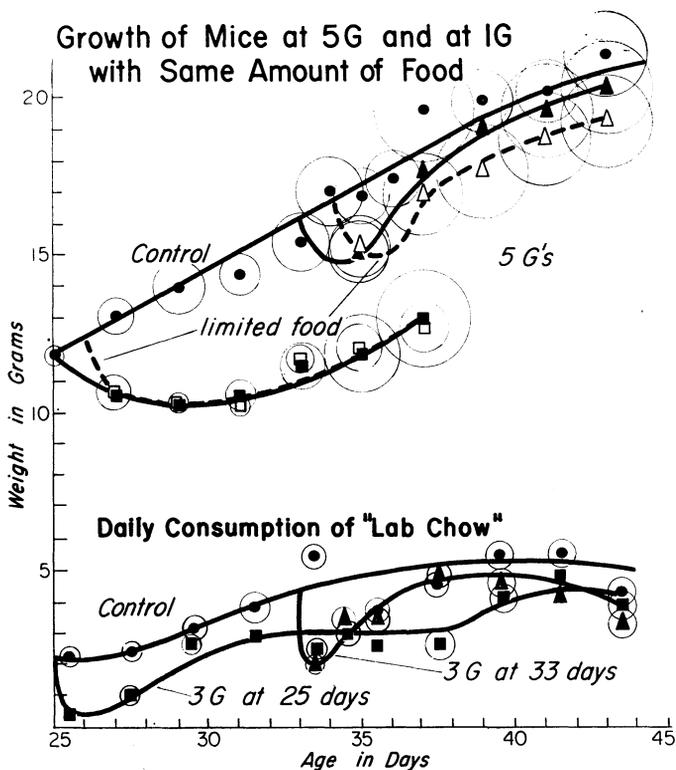source for water. The measurements of potato consumption are

Figure 2.   Broken curves are for the limited controls which were fed a diet approxi-
mately the same as that consumed by the experimental mice on the preceding
day. Standard errors are indicated by the radii of circles above or below
the experimental points. Errors for experimental "chow" consumption are
calculated by assuming their relative error to be equal to the relative,
control, standard deviation.

quite similar to the measurements for "chow" consumption. However, the measures of the former will not be reported at this time. This is because adequate corrections for the loss in mass due to evaporation have not been performed.

The mice employed in these studies are strains descended from the Swiss Webster, white strain. For the data reported in Figures 3 and 4 mice from a colony maintained by our department of bacteriology were used. These were all female mice. Twelve animals were employed in each experimental or control group. In addition to regular controls, so-called "limited controls" were employed. Those animals were fed the same quantity of "chow" and approximately the same quantity of potato consumed by the average experimental animal on the previous day.

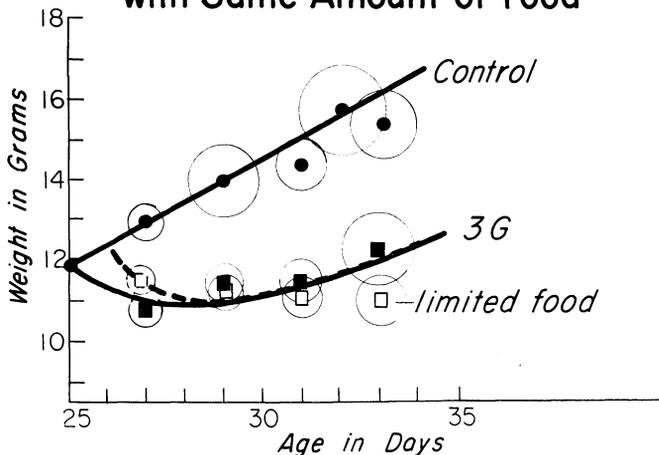## Growth of Mice at 3G and at IG with Same Amount of Food

Figure 3.   Broken lines are for limited controls.

For data reported in other figures, the NLW strain of mice was employed. In each experimental or control group approximately three animals of each sex were used. Control data are listed from average values for eight groups. Two experimental groups were employed for all mice first centrifuged at the age of three, five or seven weeks of age at fields of two, three, five, six, and seven times gravity. Other values are the averages obtained from single experimental groups.

The readers who are interested in more details about the equipment employed in this work are referred to the description of the centrifuge by Walters et al. (1960).

RESULTS AND DISCUSSION

As can be seen in Figure 2 there is a definite drop in food consumption during the first few days of centrifugation. If the field intensity is not too great, animals gradually adjust to the new environment so that the rate of food intake increases toward the control level. When this occurs positive growth resumes; the animals regain their lost mass and in most cases eventually exceed their original size.

CONSUMPTION OF "LAB CHOW"
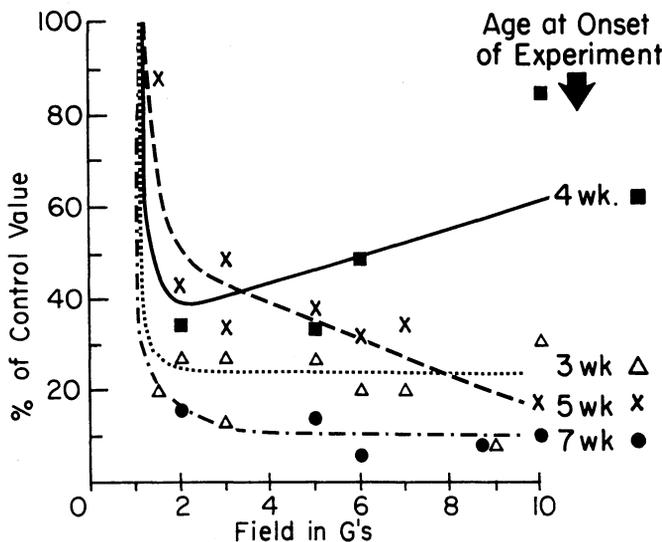**by Mice During Centrifugation**

Figure 4.   Values are for rate of food consumption during the first day of centrifugation.

Preliminary results, for mice placed on diets which approximated that of the experimental animals, are shown in Figures 2 and 3. Growth curves for these limited controls are quite similar to those for the experimental animals. Initial effects of centrifugation upon growth appear to result largely from the effect of gravity upon the animals' feeding habits.

Although increased gravity does decrease food consumption, the drop is not a linear function of the field (see Figure 4). As the field increases from normal gravity (1 G) to 2G's, the amount of food consumption drops by as much as 55 to 85 percent. Any further decrement as the field increases to 10 G's is comparatively small. However, the ability to resume normal eating habits occurs sooner at the lower fields (Figure 5).

PERIODS OF ADJUSTMENT
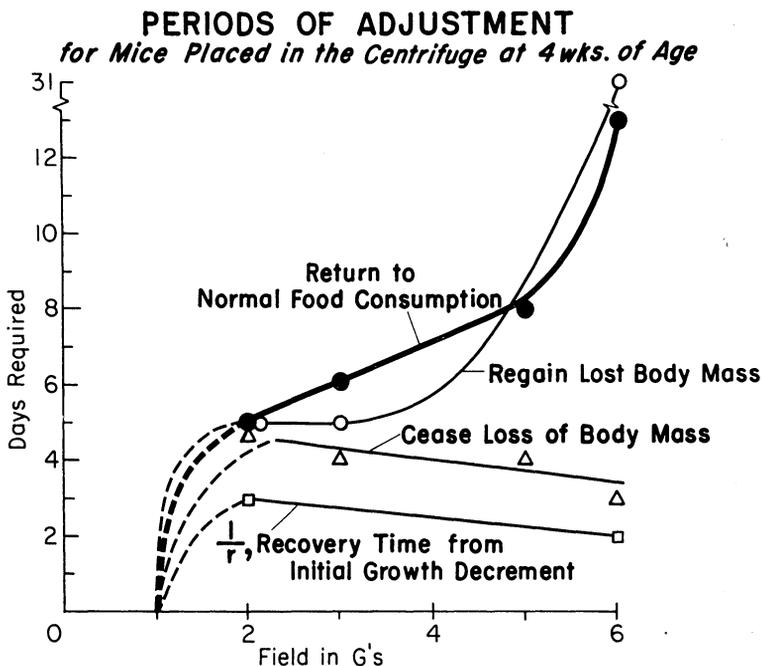### for Mice Placed in the Centrifuge at 4 wks. of Age

Figure 5. The heavier line indicates the number of days of exposure which passed before the rate of "chow" consumption rose to the level of the controls during the first day of exposure.

The seven-week-old mice demonstrate a more drastic decrement in food intake than do most of the younger mice. This is to be expected from physical considerations, larger bodies being more mechanically unstable in a given gravitational field. Contrary to these findings and expectations, the effect upon growth rate could be interpreted to be just the opposite.

A short discussion of the growth-kinetics for experimental animals is necessary in order to compare such findings with the dietary findings. Growth rates for experimental mice can be described by an empirical equation which when simplified resembles the following equation (Wunder, Wombolt, and Oberg, unpublished results):

$$\text{Growth rate} = \dot{m} = y = -Ae^{-rt} + Ye^{-lt}, \qquad (I)$$

where m is the body mass, y the experimental growth, Y the control growth rate, and t the time since the onset of exposure. The symbols A, r, and l are experimental constants whose values would depend upon the field intensity and the age at the onset of exposure. The initial decrement in growth rate is described by A, so that, at the onset of exposure, the growth rate equals Y minus A. When A is large compared to rt and lt, the

equation describes the first of the phases of growth response described in the introduction of this paper. The relative growth rate of growth constant would be equal to $\frac{y}{m}$. The initial decrement in growth constant would be $\frac{A}{m_0}$, where m_0 is the body mass at the beginning of an experiment.

This decrement in growth constant, $\frac{A}{m_0}$, demonstrates, at 6 G's, an almost linear decrease as the age for onset of exposure increases. The apparent discrepancy between this finding and the findings for food intake can be partially explained when we account for two other observations. The seven-week-old controls are consuming more food but growing at a slower rate than the younger animals. When we analyze both growth rate and rate of food intake in terms of the percent of decrement below the control level, somewhat more comparable results are obtained (Figure 6). This percentage in terms of growth would be $\frac{A}{Y}$ x 100%.

The second phase of growth response occurs when the exponent, rt, in Equation I becomes large in comparison to A. The symbol r represents an index of the recovery rate from the initial effects upon growth. The recovery time, referred to in Figure 5, is the reciprocal of r. The third phase of growth response occurs when lt becomes large. The rate at which long term,

INITIAL INFLUENCE OF 6G's UPON FOOD INTAKE
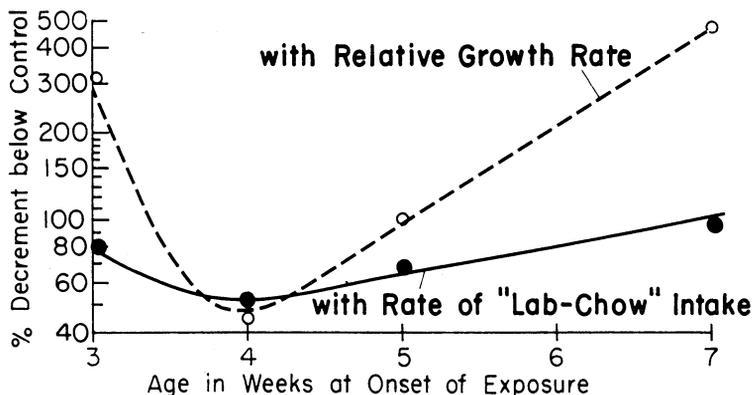### and Growth of Mice

Figure 6.   Percentages listed would be equal to 100% minus (experimental value/control) x 100%. Rates of intake are for the first day of exposure.

permanent responses set in are indicated by the magnitude of the index, 1.

We know that artifically increasing the gravitational field intensity in a mouse's environment can temporarily decrease the amount of food which an animal will eat. We believe that this change is largely responsible for changes in the animals growth. At the present time, we cannot say what changes are, in turn, directly responsible for the dietary changes. The fact that high gravity would require an animal to exert more effort during eating movements could discourage some food intake. Another factor would be the circulatory change. With some animals centrifugation causes poor venous return from the abdominal region and extremities as well as poor arterial supply to the head.

A decreased rate of venous return from the intestines would discourage the normal absorption of food materials. Experimental mice possessed intestines which were more tightly packed with food than were the intestines of the control animals. Poor circulation to the brain can result in nausea which is not conducive to a healthy appetite.

Several changes would be appropriate if this study is to be continued or repeated. One thing that is necessary is a more satisfactory measurement of the moist food and water consumed. Another change would be a measurement of food intake over a longer period of exposure and growth. A third improvement would entail the use of weighted packs which would require a control animal to support a weight comparable to the artificially increased weight of the centrifuged animal.

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