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## Growth of Turtles During Continual Centrifugation<sup>1</sup>

CHRISTOPHER H. DODGE<sup>2</sup> and CHARLES C. WUNDER<sup>2</sup>

*Abstract.* Juvenile Red Eared turtles (*Pseudemys scripta elegans*) were continuously centrifuged for a long as 9 weeks at 5 and 6 times the Earth's gravity. On a carapace-length basis growth was  $159 \pm 75\%$  more at 5 G than under control conditions (1 G). These results were contrary to those with young mice at comparable fields in which the mice displayed a slower growth rate than their controls. The nature of the response appears to be dependent upon the feeding pattern.

The effect of gravity upon organic growth is a relatively new field of study. Thus far, few detailed and long-term studies have been made. The few organisms under consideration in studies of this sort have included the bean plant (Knight, 1806), wheat cleoptiles (Gray and Edwards, 1955), fruit fly larvae (Wunder, 1955; Wunder, Herrin and Cogswell, 1959; Wunder, Herrin and Crawford, 1959), fowl (Smith, Winget and Kelly, 1959), and mice (Wunder et al., 1960). As far as the authors can determine, no long-term studies have been made with reptiles exposed to the influence of a positively altered gravitational field. It was the purpose of this study to determine whether juvenile turtles (*Pseudemys scripta elegans*) could survive in an increased gravitational field for long periods of time, and whether, under these conditions, the animals could actually survive and grow. Turtles were selected for this study because of their tough outer integument and the fact that they live in an aquatic environment.

### METHOD AND MATERIALS

Two separate experiments were undertaken. The first commenced in December, 1961; the second in February, 1962. A centrifuge, revolving at a rate of 90 rpm, was employed in this study. The centrifuge was previously described (Walters, Wunder and Smith, 1960). Turtles were kept in covered plastic refrigerator boxes (7¼" x 5½" x 3¾"), containing 500 ml. of water. Experimental turtles were subjected to from 5 to 6 G. Control animals were kept in identical containers under the same conditions of continuous light and temperature (25°C). 1961 autumn Red Eared turtle hatchlings were employed. These turtles were

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received in November and allowed to acclimatize to the aforementioned experimental conditions before exposure to centrifugation in December. This species of turtle is good for large scale studies of this sort because of its availability during all seasons of the year, its hardiness in confinement, and the relative ease with which it may be maintained and fed. The diet consisted of raw, lean, chopped beef (90%), with bone meal added (10%) and, occasionally, (every two weeks) chopped beef liver and kidney. The bone meal was used to aid in the growth and ossification of the shell. The turtles were broken down into groups as shown in Table 1.

Table 1  
First Experiment

Group	Cages	No. of Animals/Cage	Feeding	Field in G
1	2	6	daily	5
2	1	6	daily	6
3	2	6	once/3 days	5
4	1	6	once/3 days	6
5	1	6	daily	1
6	1	7	once/3 days	1
Second Experiment				
7	1	4	daily	5
8	1	4	daily	5
9	4	4	daily	1

Feeding of daily fed centrifuged turtles was accomplished during a twenty-minute period each day. At this time, the containers were also cleaned. Feeding by the animals took place both when the centrifuge was at rest and while it was revolving.

Centrifuged turtles fed once in three days were removed for a period of one day for the purpose of feeding. This was done to determine whether the altered gravity had an adverse or advantageous effect upon feeding and whether there would be a difference in the rate of growth between the two groups. Control feeding took place during the same periods as the experimental. It was necessary to clean the containers daily due to excessive fouling of water.

### RESULTS AND DISCUSSION

Growth data for both experiments are shown in Figures 1, 2, 3, and 4. Contrary to trends displayed by mice and hamsters in comparable fields, daily fed centrifuged turtles at 5 G in the first experiment grew  $159 \pm 75\%$  more than their daily fed controls (1G). This is surprising when one considers that mice at the same field grew at a rate which was only one-half that of the control mice. On the other hand, centrifuged turtles at 5 G fed only once in three days grew at almost the same rate as (actually  $28 \pm 24\%$  slower than) their controls and  $76 \pm 7\%$  less than their daily fed counterparts. The growth rate for daily fed turtles at 6 G was not significantly greater ( $46 \pm 56\%$ ) than the control growth. Results for this field must be considered as only tenta-

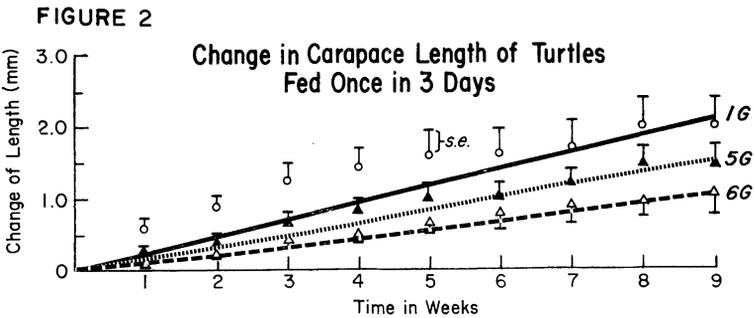
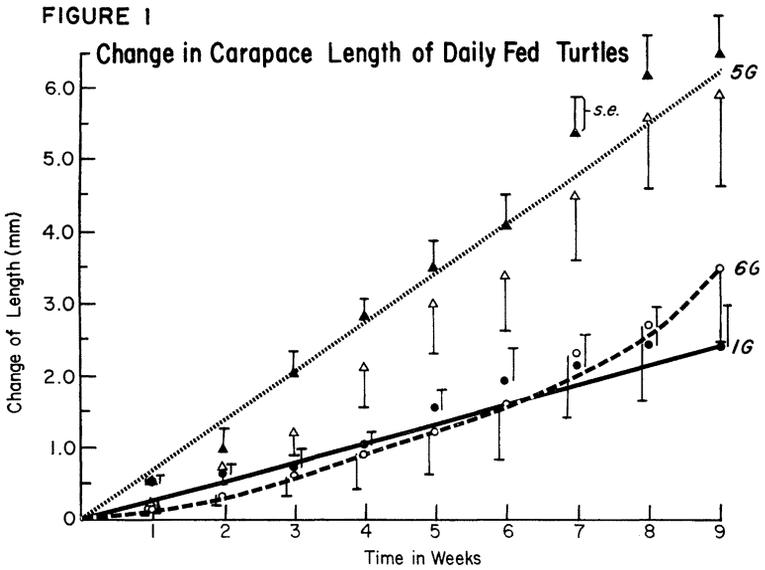


Figure 1. This figure shows enhanced growth by daily fed centrifuged turtles, especially the two groups exposed to 5 G after 9 weeks. Growth is represented by straight-line change in carapace length on a group basis. Standard errors are indicated for each value.

Figure 2. Change in Carapace Length of Turtles Once in Three Days. This figure shows a slight deceleration of growth by turtles exposed to 5 and 6 G.

tive due to mortality and illness in one cage. Turtles at this same field fed once in three days grew  $50 \pm 17\%$  less than their controls. Daily fed controls grew essentially the same amount as ( $20 \pm 37\%$  faster than) controls fed once in three days. In general, the growth trend displayed by daily fed turtles at 5 G in the second experiment was the same as that in the first experiment after 5 weeks (Fig. 3).

At 5 G, turtles grew significantly faster than the normal rate. This is surprising with respect to over-all growth trends displayed by mice in comparable fields. However, one must re-

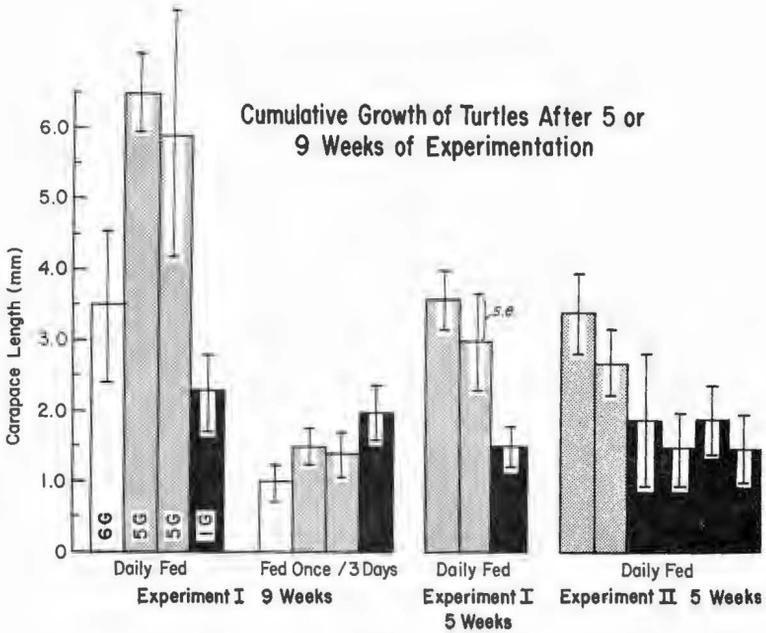


FIGURE 3

Figure 3. This figure represents total change in carapace length by all groups in Experiments 1 and 2 after 5 and 9 weeks. Similar growth trends are shown by daily fed turtles in both experiments. Standard errors for each group are indicated.

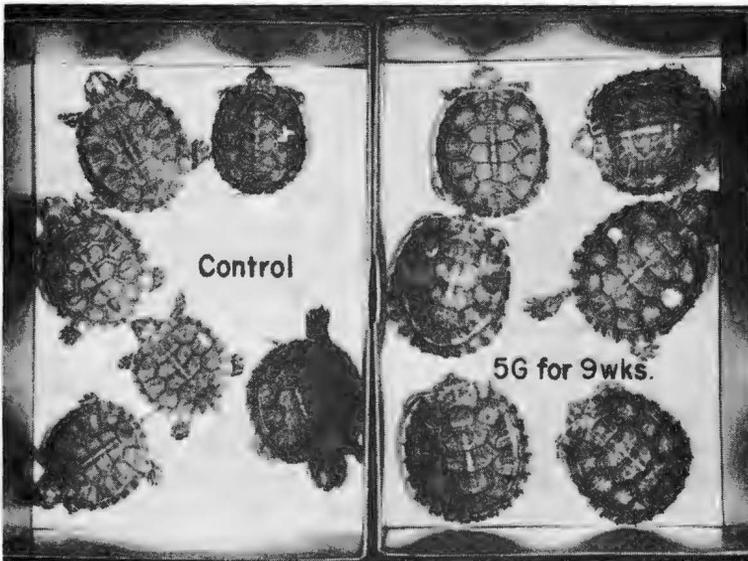


Figure 4. Enhanced Growth by Daily Fed Centrifuged Turtles at 5 G in the First Experiment after 9 Weeks of Exposure. Right: Experimental group. Left: Controls.

member that the main supporting structures of centrifuged mice (e. g., femur and gastrocnemius muscle) have been shown to increase in thickness. The fact that the shell of the turtle comprises its main support lends credence to the theory that turtles would fare better in such fields than animals with only a functional endoskeleton.

Few observations have been made on the growth of confined turtles. Cagle (1946) studied the growth of young Red Eared turtles in their natural habitat. He found that although there was a significant increment in size, especially during the first two seasons, growth was largely dependent on the physical factors of the environment during any given season. In general, this initial growth increment is reflected in our studies. However, since our experimental conditions cannot be considered normal, it would be superfluous to compare our data with those of Cagle. It must be remembered that turtles under our experimental conditions were grown during a season when turtles are normally inactive and that they were maintained under conditions of continuous light and constant temperature. However, preliminary data indicate that there may be seasonal variations in growth even under these conditions.

The accelerated growth by daily fed centrifuged turtles at 5 G is more marked than that reported for other forms. Wheat coleoptiles grew approximately 10% more during the first 24 hours of exposure to 10 G (Gray and Edwards, 1955). Fruit fly larvae demonstrated as much as a 50% increment in growth rate at 500 G (Wunder et al., 1959). There is some indication that fields of 1.5 and 2 G can accelerate mouse growth (unpublished results from our laboratory). At sufficiently high fields, growth of these forms is retarded. Apparently gravity can both stimulate and retard growth, the net response depending upon the magnitude of these two results (stimulation and retardation). Although it was found that juvenile turtles could survive in fields as great as 30 G for 36 hours both in and out of water, such a high field would be expected to retard growth after prolonged exposure.

Of interest is the fact that centrifuged turtles fed once in three days grew at the same rate or even slower than their controls and daily fed counterparts. This would seem to indicate that there is an increase in metabolic activity in centrifuged turtles and that their need for food is increased accordingly. Unfortunately the amount of food consumed by turtles was not recorded.

Some difficulty was encountered in the feeding of daily fed essentially had to take place during the time the cages were re-

centrifuged turtles. This was due to the fact that feeding necromoved for cleaning. During this twenty-minute period each day, these turtles gradually learned to gorge themselves completely before all the cages could be returned to the centrifuge and the centrifuge re-started. Therefore, it cannot be truly stated that all feeding by daily fed experimental turtles took place while the centrifuge was in motion.

Only three turtles died during both experiments. Of interest is the fact that all three deaths occurred in experimental groups during the first experiment. Autopsies revealed that the turtles had overeaten. The stomachs of these animals were so engorged with food that there was little or no remaining space for the inflation of the lungs during inspiration. Such an increased food intake upon removal from centrifugal fields has also been observed with mice and is believed to occur with fruit fly larvae.

#### SUMMARY

Growth was accelerated in daily fed centrifuged turtles. Daily fed turtles at 5 G grew  $159 \pm 75\%$  more than their controls. When the experiment was repeated, this finding was confirmed. The effect at 6 G was not so marked.

On the other hand, centrifuged turtles fed only once in three days showed, if anything, a decelerated growth. These turtles at 5 G grew significantly less than their daily fed counterparts.

Under control conditions, feeding once in three days is apparently sufficient. Daily fed control animals grew only slightly more than those fed once in three days. However, at enhanced fields more frequent feeding is necessary in order to maintain the fastest growth.

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