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SYNOPSIS. A 1-G space-based centrifuge will be necessary before controlled, bio-medical weightlessness experiments can be performed. Some argue that centrifuges of prohibitive dimensions are necessary. Labyrinthectomized, hatchling, Red-Eared Turtles (*Pseudemys scripta elegans*) which had grown to a length of 45 mm at 5 G's (200 rpm 10 cm radius) displayed the same greater shell height (21.7 ± 0.4 vs. 20.0 ± 0.3 mm at 1 G) that was observed at 5 G's with nonlabyrinthectomized, centrifuged turtles. If other effects of centrifugation can also be attributed to artificial gravity rather than rotation, a space-based centrifuge radius as short as 10 cm (1 G at 100 rpm) is suggested.

INDEX DESCRIPTORS: Turtles; gravity; centrifugation; ear; growth; labyrinthectomy.

A technique is necessary for demonstrating which of the biological effects associated with simulated alterations in gravity are actually related to the gravitational environment. Unfortunately, centrifugation not only generates artificial gravity, but also can produce nongravitational artifacts of rotation which together with gravity and sound is detected by the inner ear. Centrifuge experiments with labyrinthectomized animals are, therefore, more likely to demonstrate the effects of artificial gravity upon all other parts of the body.

The anatomy of their ear renders labyrinthectomy to be a simpler procedure with turtles than with mammals. The turtle has no external ear and the labyrinth is located directly medial to the shallow depression which is located just under the skin and which corresponds to the external auditory meatus.

Since Von Beckh (1954) performed the first experiments with a labyrinthectomized turtle, there has been little or no experimental literature on the use of labyrinthectomized turtles. Von Beckh's labyrinthectomy of *Hydromedusa tectifera* was the result of an accidental overheating and not a surgical procedure.

THE LABYRINTHECTOMY PROCEDURE

The procedure for labyrinthectomy which has been developed in this laboratory has been successfully used on some 350 Red-Eared Turtles (*Pseudemys scripta elegans*) and Box Turtles (*Terrapene carolina carolina, triunguis* and *ornata*). This simple procedure involves entering the labyrinth on both sides of the head with a dental burr and aspirating out labyrinthine tissues. Streptomycin is then used topically to assure final destruction of the sensory end-organs of the eighth cranial nerve.

After anesthetizing by an ice-water bath, the turtle's jaw is gently clamped with blunt forceps tightened by a screw with one prong bent to catch against the lingual side of the mandible. The turtle's shell is immersed in the ice-water and is secured by a burette clamp. The head is drawn forward so that either side of the head can face upward for surgery.

With the turtle thus supported and anesthetized, the initial incision is made via the external opening of the ear identifiable as an indentation into the "external auditory meatus." The ear on the Red-Eared Turtle is located 2 or 3 millimeters ventral and anterior to the characteristic red spot (or a hard oval plate at a comparable position with Box Turtles) and just posterior and slightly dorsal to the angle of the mandible.

The initial incision is made through the skin either by use of an inverted cone dental burr or by use of a flattened dissecting needle which is sharpened on one side as a miniature scalpel.

The labyrinth is then entered with an end-cutting, cross-cut fissure burr from the middle ear to which it is medial. To assure the dysfunction of the entire labyrinth it is necessary to aspirate it through a blunted and polished 18 gauge needle connected to a vacuum line followed by a second aspiration after debridement by liberal injections of Ringer's solution in the area.

A high concentration of streptomycin sulfate in Ringer's solution (one gm/liter) is then injected into the area to further assure destruction of all endings of the eighth cranial nerve. Vaseline injected through an 18 gauge needle serves as a hemostatic plug which also prevents draining of the streptomycin.

POSTOPERATIVE PROCEDURE

The turtle regains consciousness within five minutes after removal from ice and resumes feeding after 48 hours, at

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which time it may be examined for the presence of post-rotatory counterturning of the head upon spinning on a turn-table at 2 rps for approximately 5 seconds. The labyrinthectomized turtle will keep his head in the shell or just move it slightly forward. There is no vigorous postrotary head movement characteristic of normal turtles.

CHRONIC CENTRIFUGATION

In order to demonstrate that effects of centrifugation upon development are independent of stimuli to the inner ear, 6 juvenile Red-Eared Turtles (*Pseudemys scripta elegans*, shipped to us on 6-16-69 from Limberger Co., Oshkosh, Wis.) were labyrinthectomized on 7-17-69 and exposed to chronic centrifugation from 7-31-69 until 12-6-69. Their growth was compared with 6 labyrinthectomized turtles at 1 G as well as with 6 normal turtles at 1 G and 6 normal turtles exposed to comparable centrifugation (see Table I).

Centrifugation procedures were similar to those previously described (Dodge and Wunder, 1962, 1963; Wunder, 1965); but in order to enhance any rotatory stimulation of the normal turtles, 5 G's were achieved in a shorter, faster centrifuge (200 rpm with a 10 cm radius).

RESULTS

At the end of the July-to-December-1969 experiment, non-labyrinthectomized turtles exhibited definite postrotatory counterturning which was more pronounced than in any of the labyrinthectomized ones. Of the labyrinthectomized turtles two from 5 G's demonstrated no detectable counterturning, while two from 1 G demonstrated distinct counterturning and the remaining turtles showed a slight degree of counterturning, which was not characterized by the fast forceful movements of a normal turtle's head.

From reference to Table 1 it can be noted that neither labyrinthectomy, chronic centrifugation at 200 rpm, nor both prevented these animals from exhibiting significant growth. Although some turtles did not eat for a few days after surgery, the growth which ensued after 2 weeks of recovery was comparable to that of normal turtles.

TABLE 1. GROWTH OF PLASTRON LENGTH (MM) ± S.E. JULY-DEC., 1969

	Initial	*Final*	*Increment*
Lab. 1G	34.2 ± 0.6	50.0 ± 2.1	15.9 ± 1.9
Non-Lab. 1G	34.1 ± 0.4	50.6 ± 1.5	16.5 ± 1.3
Lab. 5G's	34.1 ± 0.6	47.1 ± 2.2	13.0 ± 1.9
Non-Lab. 5G's	34.4 ± 0.6	51.2 ± 4.1	16.8 ± 4.6

In this experiment the most significant effect of centrifugation upon growth was the allometric growth (Wunder, 1965, pp. 441-443) of shell height relative to carapace length (see Fig. 1). As has been previously noted in this laboratory with nonlabyrinthectomized turtles, those growing at high G are able to maintain a relatively higher shell. By the time a carapace length of 45 mm was attained, height of centrifuged animals (on the basis of distance from plastron to peak of carapace) was approximately 5% greater (see Fig. 1).

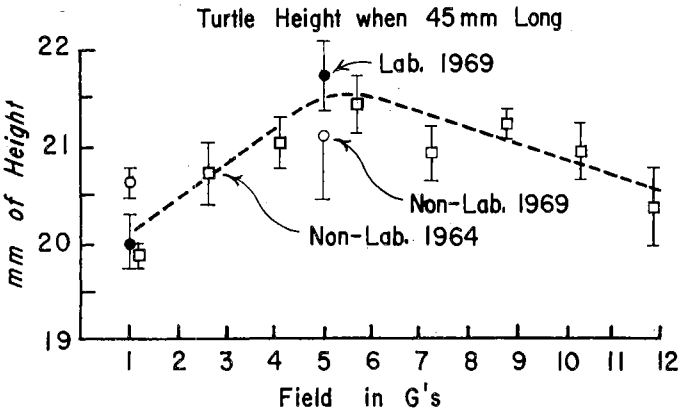

Fig. 1. Average heights obtained when carapaces achieve a length of 45 mm. The points from the present experiment are shown by circles with values for the labyrinthectomized turtle represented by closed points. Nonlabyrinthectomized values are represented by open points. Square points represent values from an experiment in 1964 using slightly different centrifugation techniques (Wunder, 1965, p. 405).

DISCUSSION

The results suggest that effects of chronic centrifugation upon shell height, at rotations as great as 200 rpm, result from the simulation of a greater gravitational force acting on the animal's body and not upon rotatory artifacts that could stimulate this animal's inner ear. The mechanism of how greater height is achieved deserves further investigation. High gravity can result in greater bone growth (Wunder *et al.*, 1960). Perhaps the ribs framing the turtle's shell accelerate the growth either in response to Wolff's Law or due to some endocrine response.

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