

## THE EFFECT OF SOMATOTROPIN AND HYPERGRAVITY ON WEIGHT BEARING EPIPHYSES OF COCKERELS<sup>1</sup>

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**ABSTRACT.** Somatotropin (STH) administered to earth gravity birds (1 g) stimulated the mean growth in height of the articular cartilage layer of the distal tibial epiphyses (DTE) and of the resting and proliferating layers of the proximal epiphyses of os tarsometatarsus (PE-TMT). However, it inhibited growth in height of the calcified cartilage zone of both epiphyses. Hypergravity control animals (2 g) showed a decreased growth in mean height of proliferating, maturing and calcified cartilage zones of the DTE as well as in the resting and calcified cartilage of the PE-TMT. Hypergravity (2 g) plus STH decreased mean height of articular cartilage zones of the DTE while increasing this parameter within the PE-TMT. The growth in height of maturing cartilage layer of the DTE and of the calcified cartilage zone of the PE-TMT was significantly decreased by administration of the hormone under hypergravity conditions.

With the exception of the articular cartilage layer of STH treated 2 g animals the mean growth in width of all cartilage zones of the DTE was significantly decreased by the hypergravity state regardless of whether the animal received somatotropin or the acetic acid suspension media (0.06M CH<sub>3</sub> COOH). STH administered to earth gravity animals significantly increased the mean growth in width of all but the articular cartilage layer of the PE-TMT. The same cartilage zones of this epiphysis showed a significant decrease in width following administration of the hormone to animals maintained under hypergravity conditions.

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### INTRODUCTION

Recent probes by orbiting satellites to the peripheries of distant planets have directed considerable interest toward the effects of increased and decreased gravity states on the skeletal frame of man and experimental animals during these missions. Centrifugation has been used in the laboratory to mimic altered gravity environments for animal and plant studies. Changes in the structure of the osseous tissue of various animals due to increased gravity states have been reported by numerous investigators (Briney et al. 1962, Negulesco 1976, Negulesco and Clark 1976, Oyama and Platt 1965, Smith et al. 1959, Smith and Kelly 1963, Smith 1975,

Wunder et al. 1960, Wunder 1962). Acute (Gauer and Zuidema 1961, Rogers 1962) or chronic (Smith and Kelly 1961, Smith et al. 1959) exposure of animals to centrifugation resulted in decreased body weight and size and structure of the musculoskeletal system (Burton et al. 1967, Oyama and Platt 1965). Femora of chronically centrifuged birds showed decreased weight (Smith and Kelly 1963) while length, weight, and diaphyseal and proximal epiphyseal diameters of non-weight bearing avian bones became smaller (Negulesco 1976). The exact cause leading to the decreased physical measurements following centrifugation remains obscure. The abated osseous parameters were considered to represent either the direct effect of the hypergravity state upon the organism (Amtmann and Oyama 1973) or to

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be the result of a partial suppression or a modification of the peripheral effects of growth hormone along with a concomitant parathyroid gland stimulation (Negulesco 1977, Negulesco 1978b).

Growth hormone administered to animals maintained at earth gravity was shown to stimulate growth of the epiphyseal plate (Asling et al. 1955) and to increase matrix production and cell replication of normal cartilage (Silberberg and Silberberg 1964, 1971; Dunn 1966, Sledge 1973). The effects of growth hormone in ameliorating or reversing the deleterious effects of hypergravity states on the articular and epiphyseal cartilage layers of long weight bearing bones have not been adequately investigated.

The present work concerns the individual and interacting effects of a 2 *g* hypergravity environment by chronic whole body centrifugation and intermittent high doses of growth hormone (STH) upon the development of cartilage zones of the distal tibial and proximal os tarsometatarsus of immature birds from 3 to 5 weeks post-hatching.

## METHODS AND MATERIALS

Healthy white leghorn cockerels were divided, at 21 days post hatching, into 4 groups of 12 animals each. The chicks were subjected, for 2 weeks, to one of the following procedures:

Group I—Earth gravity controls (1 *g*) receiving intracapsular injections into the right tibio-tarsometatarsal (T-TM) joint with 0.2 ml of 0.06M acetic acid (HAc).

Group II—Earth gravity experimental animals (1 *g*) receiving intracapsular injections into the right T-TM joint with 0.212 IU (0.2 mg) growth hormone (Porcine Somatotropin, Sigma) dissolved in 0.2 ml of 0.06M acetic acid.

Group III—Hypergravity controls (2 *g*) receiving intracapsular injections into the right T-TM joint with 0.2 ml of 0.06M acetic acid.

Group IV—Hypergravity experimental animals (2 *g*) receiving intracapsular injections into the right T-TM joint with 0.212 IU (0.2 mg) growth hormone (STH) dissolved in 0.2 ml of 0.06M acetic acid.

Animals received the treatments at 21, 24, 28 and 34 days post hatching and were maintained at 27 C, 45% humidity, and a 12-h dark, 12-h light cycle.

Animals exposed to hypergravity were placed, 3 per

cage, in cages (0.609 × 0.305 × 0.203 m) hanging from a free-swinging yoke assembly mounted on a 3.35 m radius centrifuge generating a resultant centrifugal gravitational force of 2 *g* (vectorial force parallel to the long axis of the animal) when rotated at 22 rpm. Descriptions of the structure and functional characteristics of various centrifuges for small animals are available elsewhere (Burton et al. 1967, Jankovich 1971, Kelly et al. 1960, Oyama and Platt 1965, Smith et al. 1959, Smith and Kelly 1963, Smith 1975, Walters et al. 1960). The centrifuge was stopped once daily for about 20 min to administer food, water and the intracapsular injections as required. The 4 groups of animals were given food (Purina Startina mash) and water ad libitum.

Animals were killed by decapitation at 5 weeks post hatching, 2 weeks after onset of the experimental period. The right T-TM joint was immediately removed by sectioning the adjoining bones just above or below their epiphyseal plates. Following one week fixation in 10% buffered neutral formalin, the bones were decalcified for 2–5 days in 3% nitric acid depending on their osseous density. The entire joint was doubly embedded sequentially in parlodion (2, 3 and 4%) and paraplast. The mid-sagittal region of each osseous extremity was determined to the nearest 0.05 mm with a precision caliper and the joint was serially sectioned at 7–8 μm past the established central point. The sections were stained with hematoxylin and eosin for histological examination. Three to 8 serial sections of the mid-sagittal region of each animal were read for the height and width of the articular and epiphyseal cartilage layers (resting, proliferating, maturing and calcified). Data were analyzed for significance by *t*-test (Winer 1971).

## RESULTS AND DISCUSSION

The increased mean height of the articular cartilage of a 5-week-old animal with no apparent change in columnar cyto-architecture of resting, proliferating, and maturing cartilage zones of the distal epiphysis of the tibia following administration of somatotropin to earth gravity animals suggests a selective cartilage layer growth stimulation (table 1). Urist (1972) reported that growth hormone administered to intact earth gravity animals resulted in a decreased calcification of bone and an increase in urinary calcium. In our study, the decreased mean height of the calcified cartilage layer of the distal tibial epiphyses following hormonal treatment of earth gravity animals suggests a decreased calcification. The mean height depression of the proliferating, maturing and calcified

TABLE 1

The mean height ( $\mu\text{m}$ ) of articular and epiphyseal cartilage layers of the distal tibia of chicks subjected to earth gravity and hypergravity with and without growth hormone (STH) injections.

Cartilage layer	Earth gravity (1 g)		Hypergravity (2 g)	
	0.06 M HAc	STH	0.06 M HAc	STH
Articular	2025 $\pm$ 37 (73)*	2295 $\pm$ 28 (67) <sup>††</sup>	1950 $\pm$ 34 (94)	1770 $\pm$ 31 (67) <sup>††</sup>
Resting	192 $\pm$ 6 (73)	198 $\pm$ 6 (67)	188 $\pm$ 4 (94)	192 $\pm$ 9 (67)
Proliferating	112 $\pm$ 4 (73)	112 $\pm$ 6 (67)	98 $\pm$ 3 (94)**	100 $\pm$ 8 (67)
Maturing	496 $\pm$ 12 (58)	492 $\pm$ 15 (62)	446 $\pm$ 13 (94) <sup>†</sup>	390 $\pm$ 11 (67) <sup>††</sup>
Calcified	420 $\pm$ 14 (58)	340 $\pm$ 12 (62) <sup>††</sup>	378 $\pm$ 13 (94)**	430 $\pm$ 20 (67)

\*Mean  $\pm$  SE, figures in parenthesis are numbers of samples

\*\*Significant at  $P < .05$ .

<sup>†</sup>Significant at  $P < .01$ .

<sup>††</sup>Significant at  $P < .001$ .

cartilage zones of the weight bearing tibia following exposure to the 2 g environment parallels previous work in which distal epiphyses of non-weight bearing avian radii underwent a reduction in growth following exposure to chronic hypergravity conditions (Negulesco 1978a, Negulesco and Kossler 1978).

Inhibition of skeletal growth following centrifugation was postulated to be mediated directly by the effects of the hypergravity state upon the organism (Amtmann and Oyama 1973) or indirectly by suppression or modification of peripheral effects of growth hormone (somatomedins?) with a concomitant parathyroid gland stimulation (Negulesco 1977, Negulesco 1978b). The mechanism whereby administration of growth hormone and animal centrifugation result in a decreased mean height of articular and maturing cartilage zones of the distal tibial epiphyses can not be ascertained from the present data. The marked inhibition of the articular cartilage of the distal tibia due to present treatments is especially notable since the readings were taken from the mid-sagittal region of the epiphyses. Most of the body weight and the gravitational compressing forces are supported by the condylar portion of the tibia as it articulates on the shallow surfaces on the proximal end of os tarsometatarsal. Thus, the

articular cartilage of the median sagittal section of the tibia would be least affected by the increased animal weight due to superimposed gravitational forces.

Growth hormone administered under the present conditions increased the mean height of resting and proliferating cartilage zones while inhibiting the growth in height of the calcified cartilage zone of the proximal tarsometatarsal epiphyses of the earth gravity chicks (table 2). This finding is in agreement with the known effects of growth hormone, a growth stimulation of the epiphyseal plate and an increased plasma calcium level when administered to a developing animal (Urist 1972). The overall effect of centrifugation which appears to be inhibitory in nature, is revealed by the decreased growth in height of the resting and calcified cartilage zones of the proximal tarsometatarsal epiphyses of acetic acid treated 2 g animals. This generalized epiphyseal inhibition following animal exposure to the hyperkinetic state has been duplicated in our earlier work involving epiphyseal measurements of non-weight bearing avian radii (Negulesco 1976, Negulesco and Clark 1976, Negulesco 1977, Negulesco 1978a). It suggests calcium mobilization occurs, as indicated by the severely decreased height of the calcified cartilage layer, under hypergravity conditions regardless

TABLE 2

*The mean height ( $\mu\text{m}$ ) of articular and epiphyseal cartilage layers of the proximal os tarsometatarsus of chicks subjected to earth gravity and hypergravity with or without growth hormone (STH) injections.*

Cartilage layer	Earth gravity (1 g)		Hypergravity (2 g)	
	0.06 M HAc	STH	0.06 M HAc	STH
Articular	2130 $\pm$ 70 (80)*	2220 $\pm$ 55 (44)	2040 $\pm$ 58 (91)	2475 $\pm$ 62 (57)**
Resting	336 $\pm$ 7 (80)	398 $\pm$ 16 (50)**	284 $\pm$ 8 (91)**	352 $\pm$ 14 (57)
Proliferating	100 $\pm$ 3 (80)	124 $\pm$ 7 (60)**	96 $\pm$ 3 (91)	100 $\pm$ 5 (57)
Maturing	474 $\pm$ 27 (66)	484 $\pm$ 18 (60)	482 $\pm$ 24 (91)	440 $\pm$ 19 (57)
Calcified	668 $\pm$ 16 (66)	578 $\pm$ 17 (50)**	554 $\pm$ 16 (91)**	486 $\pm$ 26 (57)**

\*Mean  $\pm$  SE, figures in parenthesis are numbers of samples.

\*\*Significant at  $P < .001$ .

of whether the animal received or lacked a superimposed hormonal therapy with somatotropin.

The increase growth in height of the articular cartilage layer of the proximal tarsometatarsal epiphyses of growth hormone injected chicks probably resulted as a response to the stress stimuli generated by chronic centrifugation and the resultant increased pressure weight presented, from above, by the nature of the articulation of os tarsometatarsal with the distal end of the tibia. This ability of connective tissue elements to respond to chronic stimulation by making adjustments in their parameters is in accordance with Wolf's law (Ham 1965).

The increased growth in width of the cartilage zones of the proximal os tar-

sometatarsus epiphyses of normogravity chicks due to administration of exogenous growth hormone is accountable (Urist 1972, Sledge 1973). In contrast, cartilage layers of the distal tibial epiphyses of normogravity birds appear refractory to the present hormonal treatment (table 3). It suggests the adjoining epiphyses of a developing fowl may be polarized in a preferential proximal-distal axis in their responses to somatotropin administered under the present experimental conditions.

It was of interest to observe that in the absence of somatotropin treatment the growth in width of the distal epiphyses of tibia was inhibited by the hyperkinetic state. The same parameter, however, taken from the weight bearing proximal epiphyses of os tarsometatarsus presented no

TABLE 3

*The mean width ( $\mu\text{m}$ ) of articular and epiphyseal cartilage layers of the distal tibia of chicks subjected to earth gravity and hypergravity with or without growth hormone (STH) injections.*

Cartilage layer	Earth gravity (1 g)		Hypergravity (2 g)	
	0.06 M HAc	STH	0.06 M HAc	STH
Articular	4319 $\pm$ 117 (37)*	4351 $\pm$ 80 (38)	3875 $\pm$ 48 (62)**	3664 $\pm$ 52 (36)**
Resting	5164 $\pm$ 67 (37)	5357 $\pm$ 93 (38)	4716 $\pm$ 44 (62)**	4482 $\pm$ 37 (36)**
Proliferating	4762 $\pm$ 103 (37)	4739 $\pm$ 105 (38)	4365 $\pm$ 40 (62)**	4089 $\pm$ 35 (36)**
Maturing	4249 $\pm$ 74 (37)	4180 $\pm$ 98 (38)	3975 $\pm$ 43 (62)**	3669 $\pm$ 39 (36)**
Calcified	3546 $\pm$ 76 (37)	3381 $\pm$ 50 (38)	3269 $\pm$ 29 (62)**	3097 $\pm$ 43 (36)**

\*Mean  $\pm$  SE, figures in parenthesis are numbers of samples.

\*\*Significant at  $P < .001$ .

TABLE 4

The mean width ( $\mu\text{m}$ ) of articular and epiphyseal cartilage layers of the proximal os tarsometatarsus of chicks subjected to earth gravity and hypergravity with or without growth hormone (STH) injections.

Cartilage layer	Earth gravity (1 g)		Hypergravity (2 g)	
	0.06 M HAc	STH	0.06 M HAc	STH
Articular	2566 $\pm$ 56 (37)*	2712 $\pm$ 53 (36)	2699 $\pm$ 45 (52)	2709 $\pm$ 66 (32)
Resting	3572 $\pm$ 118 (37)	3942 $\pm$ 82 (36)**	3865 $\pm$ 106 (52)	3186 $\pm$ 81 (32)**
Proliferating	3322 $\pm$ 108 (37)	3785 $\pm$ 89 (36) <sup>†</sup>	3646 $\pm$ 117 (52)	2955 $\pm$ 104 (32)**
Maturing	3120 $\pm$ 130 (37)	3542 $\pm$ 93 (36)**	3352 $\pm$ 129 (52)	2759 $\pm$ 61 (32)**
Calcified	2929 $\pm$ 122 (37)	3238 $\pm$ 90 (36)**	2921 $\pm$ 118 (52)	2564 $\pm$ 77 (32)**

\*Mean  $\pm$  SE, figures in parenthesis are numbers of samples.

\*\*Significant at  $P < .05$ .

<sup>†</sup>Significant at  $P < .01$ .

differences from those of control animals (table 4). The finding not only supports an epiphyseal polarity, but it also suggests a greater activity in the cell population of the proximal epiphyses (of os tarsometatarsus) in overcoming the generalized inhibitory effects of centrifugation (sudden increase in body weight and/or a constant vertical pressure, through the tibia) by responding in accordance with Wolf's law (Ham 1965).

Under the present experimental conditions, growth hormone appears to augment rather than ameliorate the depressing effects of a hypergravity state on the growth in width of the articular and epiphyseal cartilage layers of the 2 apposing epiphyses. The exact nature of this cartilage inhibition can not be ascertained from present data. It finds support, however, in the suggested direct and negative feedback mechanism at the pituitary level following administration of exogenous growth hormone. One may hypothesize on 3 possibilities: the cartilage receptor sites were already occupied by somatostatins released because of the imposed stress and, thus, unavailable to the exogenous somatotropin (Muller and Percile 1966, Muller 1971); under stressful conditions exogenous somatotropin and somatotropin-like substances have a catabiotic-catabolic rather than anabolic organic effect (Hagen et al. 1972a,b, Muller et al. 1976); and, un-

der stressful situations in a developing bird, it is prolactin rather than somatotropin that has the greater stimulating effect upon growth of cartilage of the epiphyseal plate. Present investigations involving measurement of growth hormone and prolactin levels of hypergravity exposed chicks suggest the latter may represent the correct modus operandi (Fiorindo and Negulesco 1980).

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