

OXIDATIVE METABOLISM OF HEALING AVIAN BONE FRACTURES: EFFECTS OF ESTROGEN AND HYPERGRAVITY¹

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Abstract. Two week old female Rhode Island Red chicks were subjected to closed fracture of the right radius in order to study the effect of estrogen and a 2 g hypergravity environment on post fracture metabolism of callus. The healing area showed a well formed callus after 1 week with a high degree of calcification in 2 weeks. Callus wet weight was lower in normogravity animals receiving estrogen for 1 week. Callus wet to dry weight ratios were significantly lower 2 weeks post fracture due to the increased mineralization. Mean wet weight of callus after 2 weeks of healing was 28% lower than one week control. Administration of estrogen lowered the observed wet weights. Exposure to a 2 g environment for 1 or 2 weeks tended to decrease the mean weight of callus as compared to normogravity control values, and 0.4 mg estrone produced a further decrease in wet weight. The oxidative metabolism of minced callus was significantly increased after 2 weeks of healing due to the large number of actively metabolizing cells. The estrogen treated normogravity and hypergravity exposed chicks showed significantly greater callus oxygen uptake as compared to normogravity saline injected controls at 2 weeks.

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The fracture of a bone stimulates extraperiosteal, periosteal, and endosteal cells, setting in motion a series of events that results in the formation of a blastema, callus, and ultimately a bony union at the site of the fracture. How the trauma of fracture induces fracture healing is one of the unsolved problems of bone metabolism. It is known that callus formation and bone healing can be modified by a number of physiological and physical factors such as metabolic state, stress, compression, and immobilization (Pritchard 1964). We chose to study the fracture of the avian radius in young chicks because of its ready accessibility, rapid healing and relative structural similarity to human bone. The present study was designed to compare the effects of interaction of the steroid hormone estrone (Silberberg and Silberberg 1971) with the physical stress of 2 g hypergravity, which has been shown to enhance osseous development (Wunder

et al 1960, Smith and Kelly 1963), on the post fracture metabolism of callus.

MATERIALS AND METHODS

Two week old female Rhode Island Red chicks were subjected to fracture of the right radius by digital pressure while under light Nembutal anesthesia (0.1 ml/100 g body wt). The undamaged ulna acted as a natural splint and the left radius was left intact as a control. Post-fracture, groups of birds were maintained under the following conditions:

Fracture with daily injections of 0.2 ml saline (normogravity control).

Fracture with 0.2 mg or 0.4 mg of estrone injected daily

Fracture plus a 2 g environment with 0.2 ml saline injected daily (hypergravity control)

Fracture plus a 2 g environment with 0.2 mg or 0.4 mg of estrone injected daily

Normogravity birds (4-6/cage) were maintained at 27°C, 45% relative humidity, with a 12 hr light, 12 hr dark cycle. Hypergravity exposed chicks were maintained under similar light and temperature conditions but were placed 3 to a cage on a 3.35 m (radius) modified centrifuge which rotated at 22 rpm, giving a resultant force of 2 g (Kelly *et al* 1960, Walters *et al* 1960). All birds were fed Purina Startina Mash and water *ad libitum*. The centrifuge was stopped once daily for 20 min to record body weights and for addition of food, water and administration of intramuscular saline or

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estrone injections (0.2 ml) (Parke-Davis, Theelin).

Birds were sacrificed by decapitation 1 or 2 weeks post-fracture and intact and fractured radii were immediately dissected free of soft tissue. The callus was carefully cut free from the bone under a stereomicroscope and kept in sterile plastic dishes on ice. Callus and bone sections were rapidly blotted and wet weights determined with a torsion balance. Samples were then placed in ice cold physiological saline (Baxter, Tis-U-Sol, adjusted to pH 7.0 with tris buffer) and minced with a sharp, single edge razor blade. Minced samples were placed in 1 ml of physiological saline-dextrose (100 mg%) in a 1 ml reaction vessel adapted to the YSI Model 53 Biological Oxygen Monitor. After equilibration with air at 37°C for 5 minutes, an oxygen electrode was used to measure oxygen uptake for 15 minutes. After the oxygen measurements, samples were removed from the microchamber and quantitatively transferred to a tared weighing pan. Sample plus washings were placed in a 110°C hot air oven and dried to constant weight (24 hours), allowed to cool in a dessicator, and then weighed to the nearest 0.01 mg on a semimicrobalance. Data were collated for each group and treatment and then tested for significance by analysis of variance and paired-t tests.

RESULTS AND DISCUSSION

Unilateral fracture of the avian radius is well tolerated by 2 week old chicks because the undamaged ulna acts as a

natural splint. There was no change in the birds' activity or feeding habits post-fracture and by the second day we could palpate the forming callus in the region of the break. Histological samples prepared from the fracture area, after 1 week of healing, showed a well formed cartilaginous callus with active cell division in periosteal, endosteal and extraperiosteal cells. Histological preparations made 2 weeks post-fracture showed a healing area with a high degree of calcification (fig. 1). These observations are similar to those reported by Negulesco and Eglitis (1975), who described estrogen stimulation of osteoblast and chondrocyte proliferation in fracture healing of avian bone. Enhanced callus growth and mineralization was expected in rapidly growing chicks and apparently is potentiated by the anabolic effect of the estrogen (Silberberg and Silberberg 1971).

Callus wet weight was decreased in normogravity animals receiving estrogen injections for 1 week (table 1). There was, however, no significant difference in wet weight to dry weight ratios in the

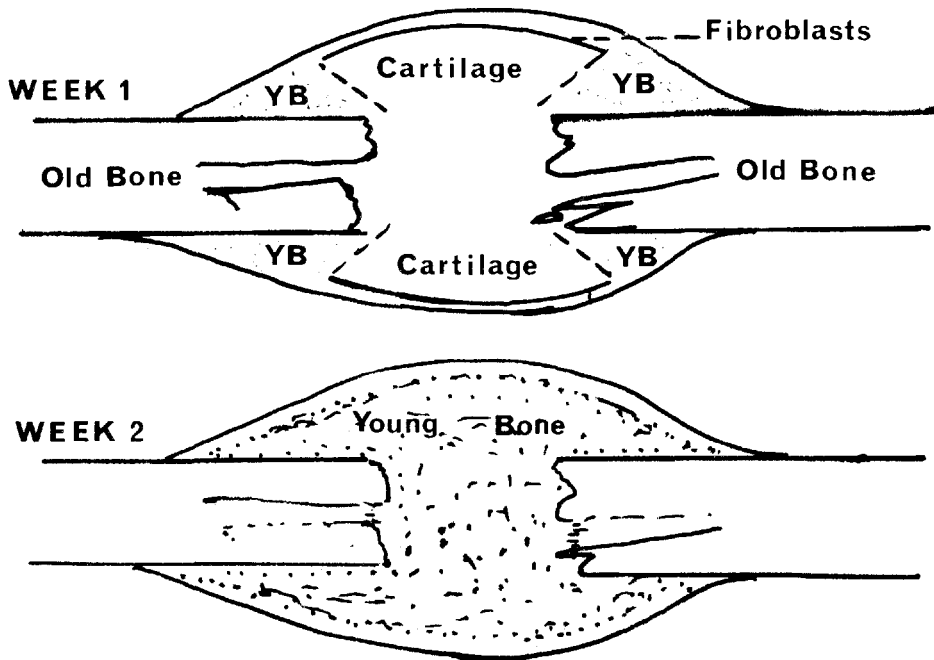


FIGURE 1. Diagram of callus and distribution of cartilage and young bone (YB) in one week and 2 week post-fracture avian radii.

estrogen treated birds. This was probably due to a concomitant slowing of mineralization during the early stages of estrogen administration, resulting in a decreased dry weight of the callus samples. When the wet weight of callus from normogravity birds was compared to that of chicks maintained at 2 g for 1 week, there was no significant difference except with the hypergravity saline injected animals which showed a 25% decrease in wet weight when compared to the normogravity saline injected controls (table 1). The observed difference cannot be attributed to the hypergravity state alone because of the general agreement of mean wet weights among the estrogen treated animals in both normo- and hypergravity conditions.

ratios were not significantly depressed by the estrogen treatment.

Exposure to a 2 g environment for either 1 or 2 weeks tended to decrease the mean wet weight of callus as compared to the normogravity control values at 1 week (table 1). The estrogen treatments had little effect on callus wet weight in the centrifuged birds except for the 2 week exposed birds receiving 0.4 mg estrone who showed a significant decrease in wet weight ($P < 0.05$). In young developing chicks, Negulesco (1977) found that 0.4 mg estrone injected daily for 1 week increased the total mass and accelerated the rate of growth of both diaphyseal and epiphyseal diameters of intact radii. He also indicated that 0.4 mg of estrone administered

TABLE 1
Comparison of Mean Wet and Dry Weights of 1 and 2 Week Post-Fracture Callus.

Daily Treatment After Fracture	Wet wt. (mg)	Dry wt. (mg)	Wet wt./dry wt. ratio
<i>WEEK 1</i>			
0.2 ml saline	66.7±5*	20.8±2	3.2
0.2 mg estrone	49.8±3	14.5±1	3.4
0.4 mg estrone	54.2±2	15.4±1	3.5
2g+0.2 ml saline	50.1±5	12.7±1	3.9
2g+0.2 mg estrone	53.5±4	15.4±1	3.5
2g+0.4 mg estrone	53.9±7	15.2±1	3.5
<i>WEEK 2</i>			
0.2 ml saline	47.8±4	21.5±2	2.2
0.2 mg estrone	43.9±4	25.1±2	1.8
0.4 mg estrone	36.0±4	19.2±2	1.9
2g+0.2 ml saline	39.7±3	20.4±4	2.0
2g+0.2 mg estrone	38.7±2	22.1±1	1.7
2g+0.4 mg estrone	29.6±4	18.6±3	1.6

*Mean of experiments ± SEM.

The callus wet to dry weight ratios, in all groups, were significantly lower ($P < 0.01$) 2 weeks post-fracture. This was undoubtedly due to the increased mineralization of the healing fracture area as indicated by the increased mean dry weight of the callus as compared to the 1 week samples (table 1). Mean wet weight of callus after 2 weeks was 28% lower ($P < 0.01$) than the 1 week samples. Administration of either 0.2 or 0.4 mg estrone further lowered the observed wet weights but the wet weight to dry

daily resulted in a greater length of fracture callus and increased growth of proximal epiphyseal diameter of fractured bones. In our series of hypergravity experiments we were unable to find a significant potentiating effect of estrogen on callus wet weight. Although there was some increase in callus dry weight of the estrogen treated 2 g exposed birds, these values were not significantly increased over the controls.

The oxidative metabolism of minced callus was significantly increased after 2

weeks of healing as compared to the 1 week samples ($P < 0.01$). This was modified only slightly by estrogen treatment (fig. 2). The observed increased oxygen uptake of callus after 2 weeks was due to the large number of actively metabolizing cells produced in the repair process. There was relatively little change in oxygen utilization by callus samples from normogravity animals 1 week post-fracture. There was, however, a general tendency for oxygen uptake of callus from estrone injected normogravity chicks to be slightly lower than saline injected controls. This effect was actually reversed in the 2 g exposed birds, but it should be remembered that the mean values obtained were not significant.

At 2 weeks post-fracture the estrogen

treated normogravity and 2 g hypergravity exposed chicks showed significantly greater callus oxygen uptake as compared to the saline injected controls (fig. 2). The effect of estrogen treatment for 2 weeks was to increase the callus oxygen uptake by 20% when compared to the oxygen uptake of callus from the saline injected controls. This estrogenic effect was not evident in the 2 g exposed birds. It is difficult to interpret the changes in oxygen uptake of callus because, to the authors' knowledge, this is the first report of this type of data. Until these experiments are repeated and expanded we would suggest that increased oxidative activity occurs whenever there is increased cell proliferation and that both hypergravity and estrogen are known to modify this parameter. The question of

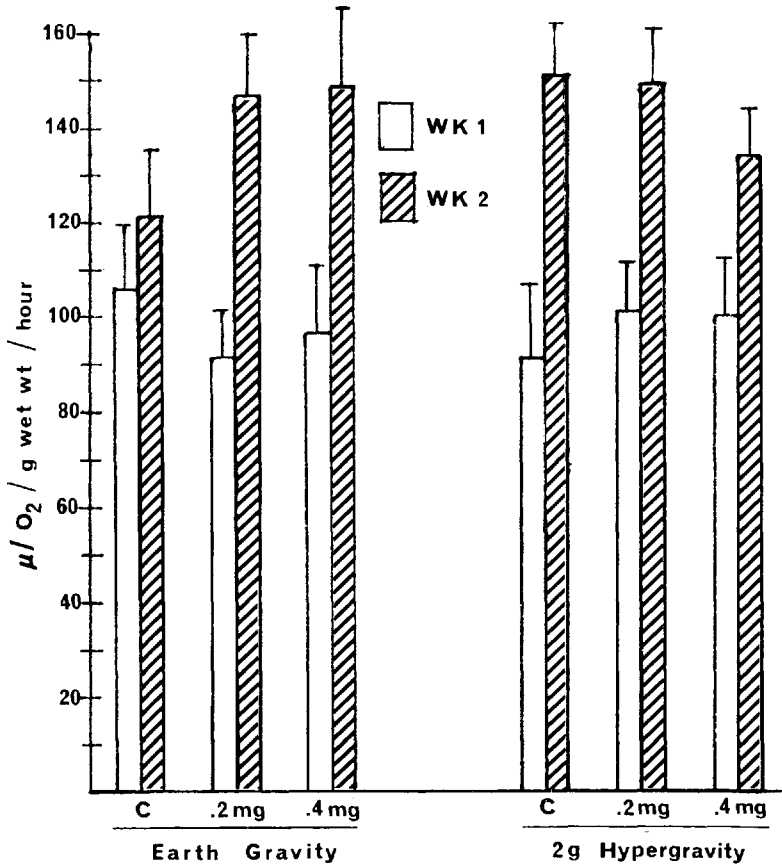


FIGURE 2. Comparison of oxygen uptake of callus from earth gravity and 2 g hypergravity exposed chicks injected with saline (C) or 0.2 or 0.4 mg estrone. These data were obtained either 1 week or 2 weeks post-fracture.

whether or not individual osteoblasts and chondrocytes increase their oxidative activity, under these experimental conditions, depends on a definitive study of the metabolism of these cells.

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