THE OHIO JOURNAL OF SCIENCE

Vol. 75 SEPTEMBER, 1975 No. 5

EFFECT OF HYPOPHYSECTOMY AND ESTROGEN TREATMENT ON LONG BONE FRACTURE HEALING OF YOUNG DOMESTIC FOWLS[1]

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ABSTRACT

NEGULESCO, JOHN A. AND JOHN A. EGLITIS. Effect of hypophysectomy and estrogen treatment on long bone fracture healing of young domestic fowls. Ohio J. Sci. 75(5): 217, 1975.

Estrogen treatment stimulates osteoblast proliferation at fracture sites of non-hypophysectomized 2-week-old birds. The hormone antagonizes growth factor of the intact 4-week bird, and induces modulation of subperiosteal osteochondrogenic connective tissue cells. It also limits neovascularization of the fracture site cartilaginous union collar of the 4-week old animal. Hypophysectomy with or without estrogen treatment results in fracture site ischaemia which depresses osteogenesis in the 2-week-old bird, and increases cartilage production at 4 weeks post hatching. Hypophysectomy without estrogen treatment decreases maturation and proliferation of connective tissue cellular elements and leads to accumulation of early osteochondrogenic cells at healing fracture sites of 4-week-old birds.

The basic problems in fracture healing are the outcome of stimulation, multiplication, differentiation, and proliferation of extra-periosteal, periosteal, and endosteo-medullary connective tissue cellular elements. The succession of these events results in the formation of a reparative blastema, callus, and bony union. Each fracture represents an unique study complicated by the nature of its highly pliable and pluri-potential cell population, differing internal environment (blood supply and oxygen tension), and a multitude of superimposing physical factors such as stress, strain, compression, and immobilization (Pritchard, 1964).

Osteogenesis within a healing fracture callus was considered by Ham (1930) to depend on the local circulation. Bone develops in a vascularized fracture while cartilage predominates in avascular or poorly penetrated sites. Ideal fracture management was shown by McLean and Urist (1968) to convert the callus to young bone. Less desirable conditions exemplified by unsplintered mobile breaks, or those traumatized by compressive and shearing forces, result in an unpredictable fracture outcome. The initial union, instead of bony may be cartilaginous or fibrous (Cohen and Lacroix, 1955).

NOMENCLATURE

In order to avoid confusion in terminology the proliferative cells of the osteogenic layer of the periosteum will be designated as osteochondrogenic. All cellular entities between mesenchymal (fibroblasts) and osteoblasts associated with osteoid seams or established cartilage and bone cells are considered as early and late osteochondrogenic cells. The proliferative subperiosteal group is labelled as "early osteochondrogenic," and the central group of cells, closer to the fracture site is presently recognized as "late osteochrondrogenic." Morphologic differences between these cells groups are clearly evident in routine histologic

[1]Manuscript received May 13, 1975, revised September 24, 1975 (#75-28).

preparations. Osteochondrogenic cells are viewed as a highly versatile population able to generate either cartilage or bone (Tonna and Pentel, 1972). Cell modulation and the outcome of a fracture, therefore, depends on the immediate conditions and the therapy influencing these cells as they circumscribe the osseous disjoint.

The present study was designed to compare, at seven days post fracture induction, the fracture site connective tissue cell population of the healing long bone in young chicks. Fracture site cell population of 2 and 4-week old birds was compared with 2 and 4-week old animals treated with the anabolic hormone (estrogen), or pituitary ablation, and a combination of both procedures.

MATERIALS AND METHODS

Male and female Rhode Island Red chickens of 2 and 4 weeks of age were exposed to complete fracture of the right radius by digital pressure under Nembutol anesthesia. Animals of each age group were subjected to one of the following procedures: (A) fracture only (controls), (B) fracture followed by estrogen injections, (C) prefracture hypophysectomy without estrogen treatment and (D) prefracture hypophysectomy with estrogen administration. After treatment the chicks were divided by age group and maintained in a temperature humidity controlled avian room. The 2-week-old animals were maintained 10 to a cage (3′×3′×1½′) at 80 to 90°F. The larger, 4-week-old birds were placed four to a cage in wire cages (2′×2½′×1½′) at 72°F with a 12 hour light, 12 hour dark cycle. Relative humidity of the aviary was maintained at 45%–55%. All animals were watered and fed at libitum with Purina-Startina mesh.

Oral approach to the pituitary gland was employed in the ablation procedure. The gland was exposed by cutting the soft tissue on the roof of the mouth with a cauterizing needle then drilling the bony elements under the gland with a dental drill and burs (no. 2–6). Bleeding, where present, was controlled by: Gauze sponge, cotton swabs, gelfoam (Upjohn), suction, and gentle freezing with crochet needle previously cooled on a CO_2 block. The gland was cut free with sharp dental probes and removed with a fine-tip spatula. The site of the gland was cauterized with an ophthalmological cautery apparatus. The osseous deficiency under the sella trucica was sealed with melted Baseplate Truewax. Hypophysectomized animals were examined at the end of the experiment by dissection and sectioning to note the success of the operation procedure. Fracture of the right radius, and onset of estrogen injections began after a 24-hour post-operative rest period. The fractured bone was set in place by digital pressure and the normal ulna acted as a natural splint for the fractured bone. Hormonally treated animals were injected daily in the posterior thigh musculature with 0.1 mg, 1000 IU/100 g of body-weight Esterone suspended in saline solution (Theelin, Parke-Davis) for 7 days and control animals were injected with saline only.

Chicks were sacrificed by decapitation after one week of healing. The right radius was removed immediately after sacrifice, dissected free of soft tissue, fixed in buffered formalin, and decalcified for 2–5 days in 3% Nitric acid depending on the density of the bone. The radii were doubly embedded (2, 3 and 4% parlodion and paraplast), sagittally sectioned at 4–5 μm, and routinely processed with hematoxylin and eosin for histological examination. The data reported in figures 1 and 2 depict the mean cell population of 15–20 oil immersion fields per section from 10 serial sagittal sections per callus. The fields for cell counting were taken along the greatest transperiosteal height of the healing fracture callus. An attempt was made to observe the degree of fracture site neovascularization. Fracture sites were considered well supplied when invaded by 15 or more new vascular beds, intermediate between 10 and 15, and poorly vascularized at less than 10 vascular beds. The data were analyzed statistically by an F test one way analysis of variance and t test.

RESULTS

Estrogen administration increased long bone fracture site mesenchymal cell population at 2 weeks post-hatching. This cellular response following hormonal treatment appeared independent of an intact or extracted hypophysis at this animal age (fig. 1). Increased numbers of mesenchymal cells were noted at fracture sites of 4-week-old chicks when the prefracture hypophysectomized animal received estrogen treatment. Mesenchymale cell population was decreased at fracture sites of 2-week-old prefracture hypophysectomized animals lacking estrogen treatment and in the 4-week-old prefractured animal receiving estrogen or without hormonal treatment with a prefracture hypophysectomy.

Fracture followed by estrogen treatment increased and revealed a highly significant difference ($p<0.001$) between early and late osteochondrogenic cells of the non-hypophysectomized 4-week-old bird population (fig. 2) as compared to normal animals. The procedure had little or no effect upon the fracture site cell population of early and late osteochondrogenic cells of the 2-week-old animal. Prefacture hypophysectomy without es-

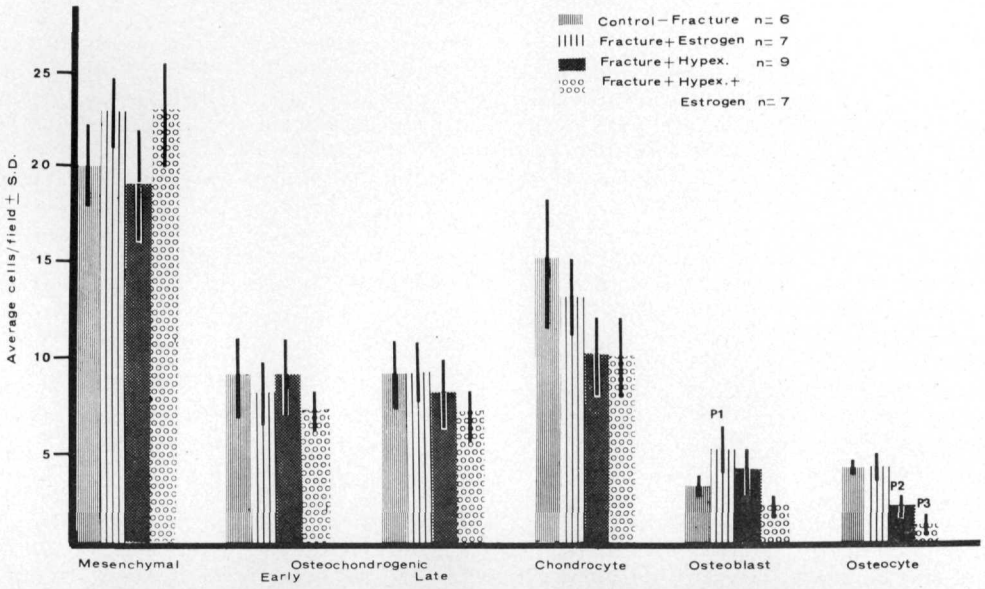

FIGURE 1. Comparison of transperiosteal fracture site mean cell population per oil immersion field of control and experimental 2 week old birds at 7 days post fracture induction. P1=p<0.05, P2=p<0.01, P3=p<0.001. The bars indicate the standard deviation of the mean.

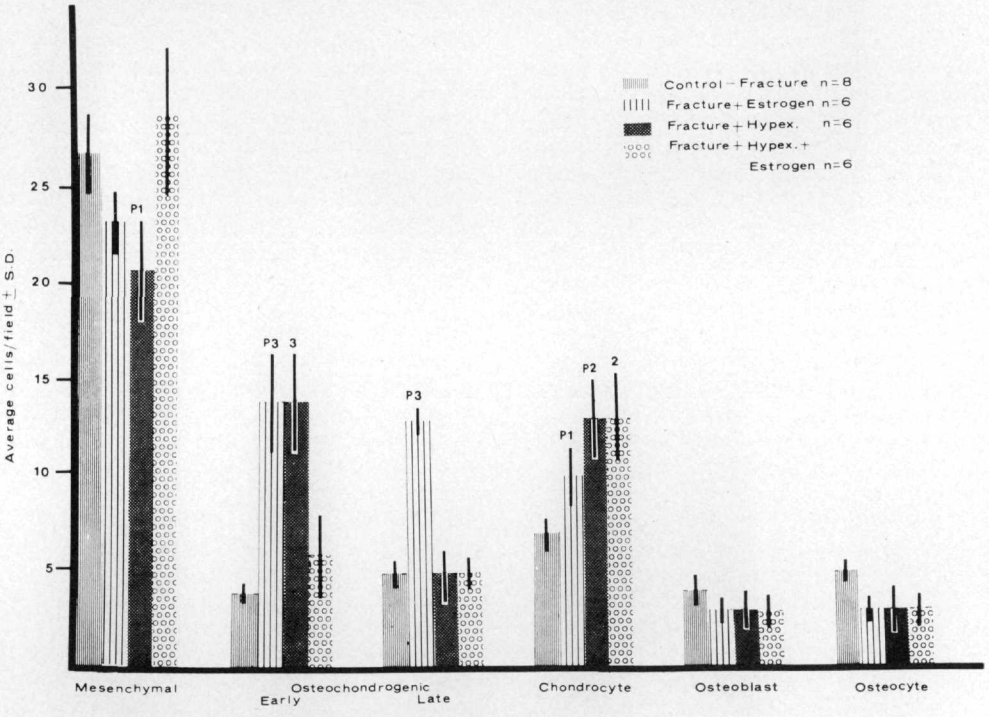

FIGURE 2. Comparison of transperiosteal fracture site mean cell population per oil immersion field of control and experimental 4 week old birds at 7 days post fracture induction. P1=p<0.05, P2=p<0.01, P3=p<0.001. The bars indicate the standard deviation of the mean.

trogen treatment significantly increased the early osteochondrogenic cell population ($p<0.001$) of the 4-week-old chick (fig. 2.) This procedure did not affect the early osteochondrogenic cells at 2 weeks post-hatching or the late osteochondrogenic cellular compartment of either the 2- or 4-week-old animal.

Estrogen administration increased the fracture site chondrocyte population of prefractured, non-hypophysectomized, 4-week-old animals ($p<0.05$, fig. 2). It had no significant influence, for the same procedure and cell type, at 2 weeks post-hatching. Prefracture hypophysectomy either with or without estrogen treatment increased fracture site chondrocyte population of the 4-week-old animal ($p<0.01$, fig. 2). These chondrocyte responses were not observed at healing fracture sites of prefracture hypophysectomized 2-week-old animals either supplied with or lacking estrogen treatment.

Estrogen administration increased the osteoblast population at healing fracture sites of normal or non-hypophysectomized animals at two weeks post hatching (fig. 1). This effect was not observed in 2-week-old birds with a prefracture hypophysectomy or in the 4-week-old chicks. Prefacture hypophysectomy significantly depressed fracture site osteocyte cell population of the 2-week-old animal group ($p<0.01$). This cell depression was most severe ($p<0.001$) when the prefracture hypophysectomized animal was supplemented with the hormonal treatment (fig. 1). Osteocyte responses for the non-hypophysectomized 2-week-old bird or for all experimental groups at 4 weeks post-hatching were statistically insignificant.

Decreased neovascularization was observed in the histologic examination of fracture sties of all hypophysectomized 2-week-old birds. Vascular invasion into fracture site was most severely depleated when the prefracture hypophysectomy procedure was followed with the estrogen treatment. Fracture sites of control 4-week-old birds demonstrated the largest degree of neovascularization. There was a small or intermediate degree of new blood vessel invasion into fracture site regions of estrogen treated non-hypophysectomized birds at 4 weeks post-hatching. Neovascularization was greatly limited at fracture sites of prefracture hypophysectomized 4-week-old chicks either supplemented with or lacking estrogen treatment. This vascular response was more limited than in the non-hypophysectomized 4-week-old bird receiving the estrogen treatment. Fracture sites of both control and experimental 2- and 4-week-old animals showed various degrees of cartilaginous union. No fracture site resulted in complete osteosynthesis at one week post fracture induction.

DISCUSSION

Cellular differentiation, matrix synthesis, calcification and ossification in the fracture healing of long bones in young birds may be controlled by changing the hormonal environment of the animal. The removal of the pituitary gland of the bird has been shown to exert profound effects on long bone structure (McWhinnie and Thommes, 1973). Effects of estrogen upon connective tissue cellular elements have been extensively studied and reviewed (Schiff, 1966, Silberberg and Silberberg, 1971). The role of ablation of the pituitary gland and estrogen treatment upon fracture healing of young birds either alone or combined has never been clarified. Our data indicate that the presence of prefracture hypophysectomy alone or when supplemented with a superimposed estrogen treatment decreases and delays fracture healing of long bone in young birds.

The increased osteoblast population observed at fracture sites of estrogen treated non-hypophysectomized 2-week-old birds (fig. 1) suggests a potentiating hormonal effect upon this cell type in long bone fracture healing. Osteoblast proliferation has been previously reported in non-fractured avian bones by Bloom et al. (1941), Landauer et al. (1939), and Landauer and Zondek (1944). Healing fracture sites of hypophysectomized non-estrogen treated 2-week-old birds showed decreased osteocyte populations and ablation of the pituitary gland apparently depressed the healing abilities of 2-week-old birds. Thus, decreased maturation and proliferation of both pre-cartilaginous and pre-osseous connective tissue cellular elements was

observed in the absence of the pituitary. Retardation of avian skeletal growth due to hypophysectomy has been reported by Thommes et al. (1973). Decreased neovascularization was observed in histological examination of fracture sites in all of our hypophysectomized 2-week-old animals. Ablation of the pituitary gland not only depresses cellular activities and body growth as shown by McWhinnie and Thommes (1973), but hypophysectomy may also be responsible for the greatly limited neovascularization observed at fracture sites of these animals. It is believed that the limited, week-long healing period selected for this study did not allow sufficient time for adequate new vascular penetration into the fracture site. Greatly limited neovascularization results in ischaemia and fracture site ischaemia favors formation and persistance of a cartilaginous callus. This, in turn, results in depressed bone formation as observed by previous investigators (Girgis and Pritchard, 1958).

Estrogen treatment appears to be another factor which greatly depleates osteocyte population at fracture sites of prefracture hypophysectomized 2-week-old birds. This bone cell depletion may be attributed to hormonal effect in delaying neovascularization of the fracture callus. The net result is decreased cartilage erosion, and longer persistance as suggested by Ham (1930) and Silberberg and Silberberg (1971). Thus, it is apparent that some unfavorable conditions for osteogenesis such as ablation of the pituitary gland, limited healing time, and a negative estrogenic effect upon neovascularization favor fracture site cartilage and not bone formation.

The apparent delay in cell maturation, and subsequent increase in the osteochondrogenic cell population at fracture sites of non-hypophysectomized estrogen treated 4-week-old bird is believed to be due to the estrogenic effect on stimulating the onset of modulation of undifferentiated connective tissue cellular elements as suggested by Schiff (1966). Increased early osteochondrogenic cell numbers in the prefracture hypophysectomized 4-week-old bird without estrogen treatment (fig. 2) can be attributed to a decreased cellular maturation and proliferation process. This may have been due to the hypophysectomy procedure which results in an accumulation of immature or early preosseous and precartilaginous cell forms at fracture sites of these animals. Estrogen treatment and prefracture hypophysectomy appear to inhibit rather than enhance the early osteochondrogenic cell population. It can only be suggested that this finding is correlated with and reflected in the increased mesenchymal cell population for this procedure at this particular animal age.

Increased chondrogenesis, as evidenced by high chondrocyte counts, dominated the histologic picture of the 3 experimental groups of 4-week-old birds (fig. 2). Estrogen treated 4-week-old non-hypophysectomized birds had more cartilage formation than the controls, but this was less than that observed with the prefracture hypophysectomy, either with or without estrogen treatment (fig. 2). Histologic examination of fracture sites of control 4-week non-hypex birds revealed a higher degree of new blood vessel invasion of the osseous disjoint than in the experimental animals. This was probably due to the intact pituitary which allowed for development of an adequate neovascularization.

There was a small or intermediate degree of new blood vessel invasion into the fracture sites of estrogen-treated non-hypophysectomized 4-week-old chicks. In this particular animal group we were, perhaps, observing a mutual antagonism between estrogen and growth factor of the bird. Evans and Simpson (1931) and Simpson et al. (1950) showed that estrogen and avian growth factor appear to cancel each other's effect on chondrogenesis within the fractue callus, and together result in an intermediate vascular response. If cartilage multiplication, proliferation and destruction were somewhat delayed because of estrogen treatment, then the estrogenic effect upon cartilage was decreased due to an intact pituitary gland and resulted in better neovascularization within the cartilaginous collar.

Neovascularization at fracture sites of hypophysectomized 4-week-old chicks either supplemented with or lacking estrogen treatment was greatly limited. Their fracture sites showed the greater cartilage

production (fig. 2). There was no difference in the chondrocyte population of the cartilaginous callus of the 4-week-old hypophysectomized animal, either with or without estrogen treatment. An animal already maximally depressed by removal of the pituitary and possessing a fracture site environment predisposed to form cartilage appears unlikely to be further suppressed by the estrogen treatment.

The observed mesenchymal cell depletion at fracture sites of prefracture hypophysectomized 4-week-old chicks without estrogen treatment remains unexplained. It is interesting to note, however, that mesenchymal cell counts were slightly depressed, but not significantly so, by hypophysectomy at 2 weeks post hatching.

The present study indicates that estrogen treatment enhances fracture site osteogenesis by increasing the osteoblast population in the non-hypophysectomized 2-week-old chicken. Estrogen also induces osteochondrogenic (early and late) and cartilage cell accumulation at fracture sites in non-hypophysectomized 4-week-old birds. Hypophysectomy was shown to have an adverse effect upon fracture healing of long bones in young 2- and 4-week-old birds regardless of whether the animal is given an estrogen treatment. Hypophysectomy decreases osteogenesis at fracture sites of 2-week-old animals and results in cartilaginous unions of fractured long bones at 4-weeks post-hatching.

Acknowledgments. This investigation was supported, in part, by General Research Support Grant Number 5409, National Institute of Health. The writers would like to thank Dr. J.M. Delphia for reading the manuscript and Ms. M.E. Amspaugh for the typing.

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