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# The Use of Xeroradiographic Imaging to Evaluate Fracture Repair in Avian Species

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**Abstract.** The purpose of the study was to determine how fracture healing occurs in avian species and to document if there are any major differences between medullary and pneumatized bone repair. The avian skeleton with both types of bones in a single species affords an opportunity to evaluate the importance of hemorrhage and bone marrow in the fracture healing process. The general concept of fracture healing in humans is well documented but certain specific aspects of fracture repair remain unclear. Two of these issues are the importance of hemorrhage and the influence of bone marrow in fracture healing. Utilizing correlative studies, we wished to determine if xeroradiographic images reflected histologic changes as healing occurred and to determine if this form of radiographic imaging provides an appropriate monitoring study to assess progress of fracture repair in the clinical circumstance.

Correlating the xeroradiographic images with the histologic findings evident of fracture healing was discerned before the actual callus formation occurred. Xeroradiographic imaging was quite helpful in visualizing callus formation and was found to be superior to plain radiographic studies.

**Key words:** Xeroradiography – Histology – Fractures – Hemorrhage – Bone marrow.

The anatomy and structure of the avian bone differs from that encountered in mammals [5, 12, 18]. In a number of avian species the proximal long bones are well pneumatized and bone marrow is replaced with diverticula that communicate with the air sacs of the respiratory system [5, 12, 16, 18]. The large pneumatized medullary cavity has sparse trabeculae. The cortical bone is also thin and brittle due to a high calcium content which results in a decrease in tensile strength [5, 12]. Differences in the structure of avian bone pose fundamental questions regarding the process of avian fracture healing [13, 16, 19]. The time course of avian fracture repair in pneumatized versus non-pneumatized bone is not known.

Although we have a general concept of fracture healing in humans, certain specific aspects of this repair remain unclear [9, 11]. At some issue is the importance of hemorrhage and the influence of bone marrow on fracture healing. The purpose of this study was to determine how fracture healing occurs in avian species and to document if there are any major differences between medullary and pneumatized bone repair. We believe that the presence of both types of bones in a single species affords an opportunity to evaluate the importance of hemorrhage and bone marrow in the fracture healing process. Additionally, by correlative studies we wished to determine if xeroradiographic images reflected the histologic changes as healing occurred and to determine if this form of radiographic imaging provides an appropriate monitoring study to assess progress of fracture repair in the clinical circumstance [15].

## Materials and Methods

Domestic pigeons were used because they have both pneumatized bones and well developed bone marrow spaces. Each of the 24 subjects was given 30 mg intramusculary (I.M.) of ketamine HCl.[a] When an appropriate level of anesthesia for surgery was obtained, the humerus in one wing and the ulna or radius and ulna in the opposite wing were manually fractured at the mid shaft. The fracture sites were serially imaged using xeroradiography [15].[b]

[a] Vetalar, Park-Davis & Co., Detroit, Michigan
[b] Xerox Corporation, Pasadena, California

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We made no attempt to reduce, immobilize, or otherwise splint the fractured bones [1, 16, 19]. The pigeons tolerated the surgical procedures well and had adequate voluntary food and water intake. None of the pigeons appeared distressed at any time throughout the study. Each pigeon was treated by a method used clinically to treat naturally occurring fractures, which included cage rest, warmth provided by heat lamps, and a high level of nutrition [12, 16, 19]. Images were obtained immediately following the experimental fractures and at 24 h, 5, 9, 16, and 21 days, as well as at 4, 6, and 12 weeks. The birds were euthanized at the same intervals in which the images were made. Fracture sites were dissected and along with the normal contralateral bone, fixed in 10% buffered formalin, decalcified in Decal[c], embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin (Hand E). Selected sections were also stained with Gomori's trichrome and Alcian blue. Correlation was made between the xeroradiographic images and the histologic findings.

## Results

Radiographs obtained immediately after fracturing the humerus showed subcutaneous or soft tissue emphysema at the fracture site (Fig. 1). After 24 h, emphysema was no longer apparent on the xeroradiographic images. On the 24 h images, the pneumatized cavity of the humerus near the fracture site demonstrated an increased density (Fig. 2A). This was also seen in the ulna and radial fractures to a lesser extent. A radiographic density was also seen to extend into the surrounding soft tissue adjacent to the fracture site. The loss of radiolucency both in the bone and soft tissue was believed to be due to localized hemorrhage. This was confirmed by histopathologic correlation (Fig. 2B). The soft tissue areas of the humeral fracture site, at five days, appeared more radiodense than at 24 h. Histologically dense connective tissue was seen proliferating from the endosteal areas of the cancellous bone. The periosteum appeared thickened and subperiosteal bone formation was initiated along the cortical surfaces. The radius and ulnar fractures, at five days, demonstrated marked periosteal and endosteal bony proliferation, and fibrous connective tissue filled the space between fractured ends (Fig. 3A and B). Xeroradiographs of the ulna, nine days following fracture, showed prominent radiodense areas in the medullary space adjacent to the fractured ends as well as in the periosteal zones (Fig. 4). Complete osseous bridging or periosteal callus was not radiographically apparent. Histologically, the entire callus consisted of three components.

1. Cancellous bone, the mineralization of which could not be determined on decalcified sections. This cancellous bone arose at the angle formed by the bony cortex and the raised periosteum distal to the fractured ends (Fig. 5A). Bone structure formation was more extensive in the endosteal callus (Fig. 5B). Its location corresponded to the radiodense areas present on the xeroradiographic images (Fig. 4B).

2. Cartilaginous tissue which was more extensive in the periosteal areas overlying the fracture sites and not as prominent in the area of endosteal callus on the Alcian blue stain (Fig. 5C).

3. Fibrous connective tissue which occurred mainly at distal points such as the outer periosteal layer or in fractures with fragment displacement due to excessive motion at the fracture site.

In humeral fractures in which there was significant displacement and movement, the majority of the callus was still fibrous connective tissue at 16 days (Fig. 6A) with an incompletely formed bony callus and minimal cartilaginous tissue formation (Fig. 6B). Xeroradiographs at 16 days post-fracture revealed a nondisplaced ulnar fracture with a uniting callus over the periosteal surface (Fig. 6A) which histologically was mainly cancellous bone (Fig. 7A and B) with a small amount of cartilage on the periosteal side. The humeral fractures at 16 days, particularly those having significant displacement and/or movement showed a callus comprised mainly of fibrocartilage. After 21 days, xeroradiographs of the ulnar fractures demonstrated uniting callus formation which histologically consisted of cancellous bone incorporating fracture ends. In humeral fractures in which the bone shafts overlie each other, callus formation at 21 days resembled that of the ulnar fracture at nine days. By four weeks a radiodense uniting callus was present in the stable ulnar fractures. Histologically this callus consisted of dense cancellous bone. The fracture segments were well incorporated, and early osseous remodeling appeared to be present. Displaced humeral fractures contained callus of cancellous bone with dense fibrous tissue bridging the fracture site.

By six weeks, in the ulnar fractures, there was radiographic evidence of bony callus uniting the fractured ends and apparent remodeling of bony cortices. Histologically, the bone was of the cancellous type but appeared to contain thicker bars that had marrow spaces with bone marrow elements. The endosteal, periosteal, and fracture segments of cortex were contiguous. Remodeling of the original cortical shafts with reestablishment of the medullary canal appeared to be taking place. In the humeral fracture after 16 weeks, the histologic appearance of the callus showed that the poorly approximated and aligned fractures were unchanged from their appearance at 16 days (Fig. 8). By contrast, the well aligned fracture showed a decrease in callus density from 6 to 12 weeks which was interpreted to be due to remodeling of the callus after solid osseous union had occurred. Despite anatomical differences, fracture repair appeared to follow the same general processes as that seen in mammals.

[c] Reg. TM of Omega Chemical Corp., Cold Spring, NY

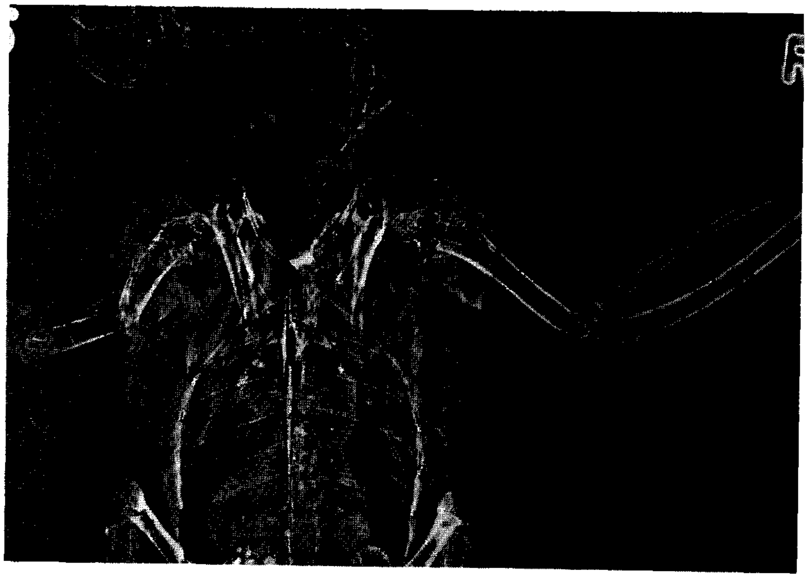
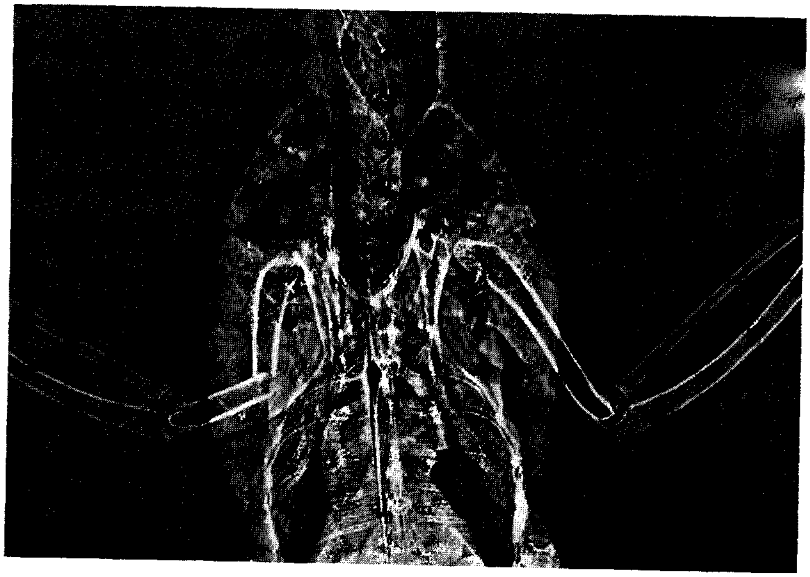


**Fig. 1.** Xeroradiograph (negative mode) of fractures of pneumatized (humerus) and medullary (ulnar) bone. Minimal emphysema is seen in the area of the left humeral fracture. At this moment only minimal displacement of the fracture fragments is noted. The right humeral cavity is more radiolucent than the left



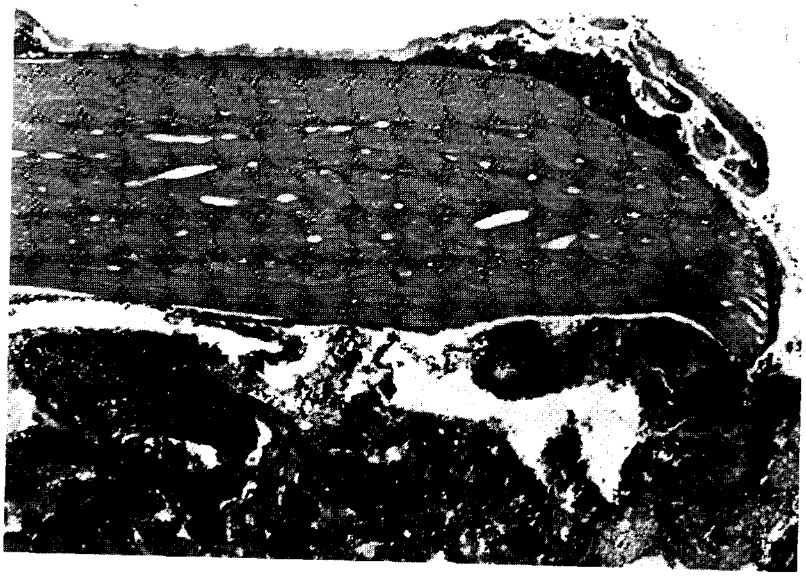
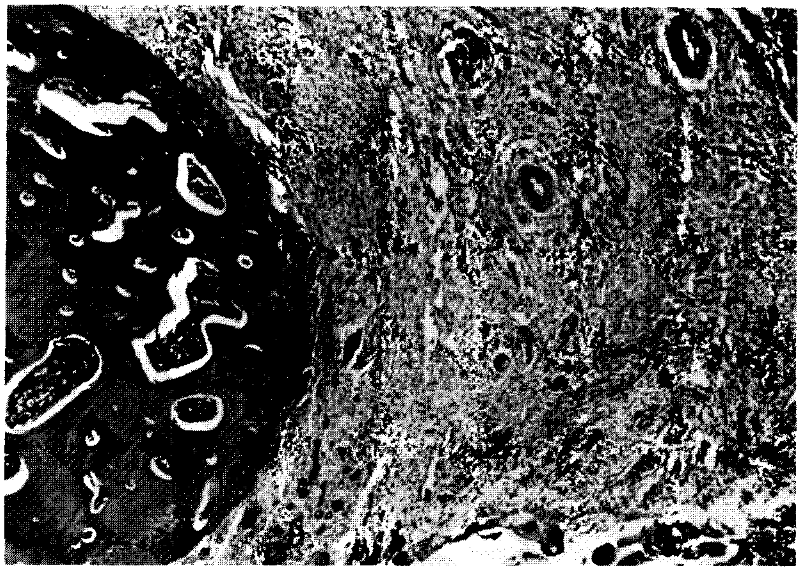


**Fig. 2. A** Xeroradiograph (negative mode) post-fracture in another bird shows no definable soft tissue emphysema in the area of the humeral fracture. The disparity in radiolucency of the humeral cavity when compared with the contralateral side is again noted
**B** Histologic section of humeral fracture site at 24 h (H and E, ×6.3). Hemorrhage within the bone marrow cavity is present. This correlates with the decrease in humeral cavity radiolucency noted on the xeroradiographs. (Adapted from Healing of Avian Fractures. J. Am. Anim. Hosp. Assoc. 16: 768–773. Bush, Montali, Novak and James, 1976)



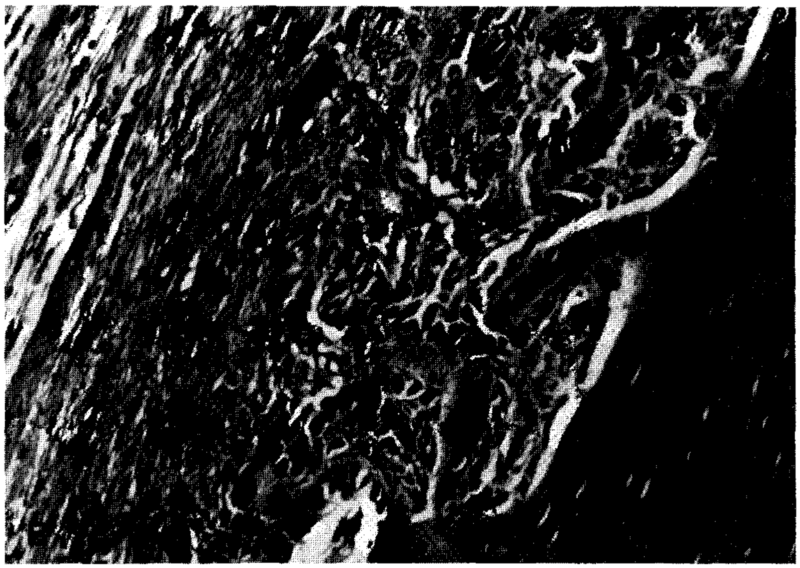


**Fig. 3A and B.** Histologic sections (H and E, ×6.3) of the radius and ulnar fractures at five days. Periosteal and endosteal bony proliferation is noted. Fibrous tissue fills the space between the fractured segments

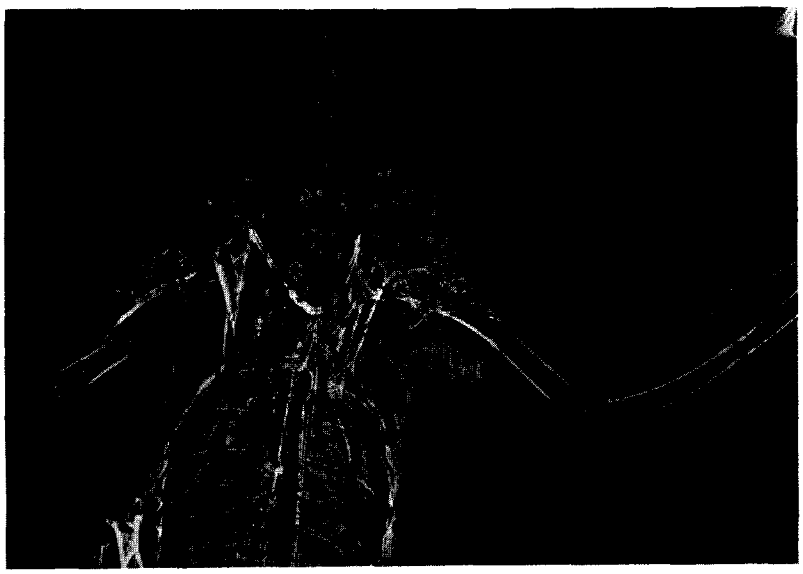


**Fig. 4.** Xeroradiograph (negative mode) nine days after fracture. The humeral fracture is displaced and no evidence of osseous or fibrous bridging is seen. Evidence of periosteal reaction, cortical thickening and osseous sclerosis is noted in the area of the ulnar fracture

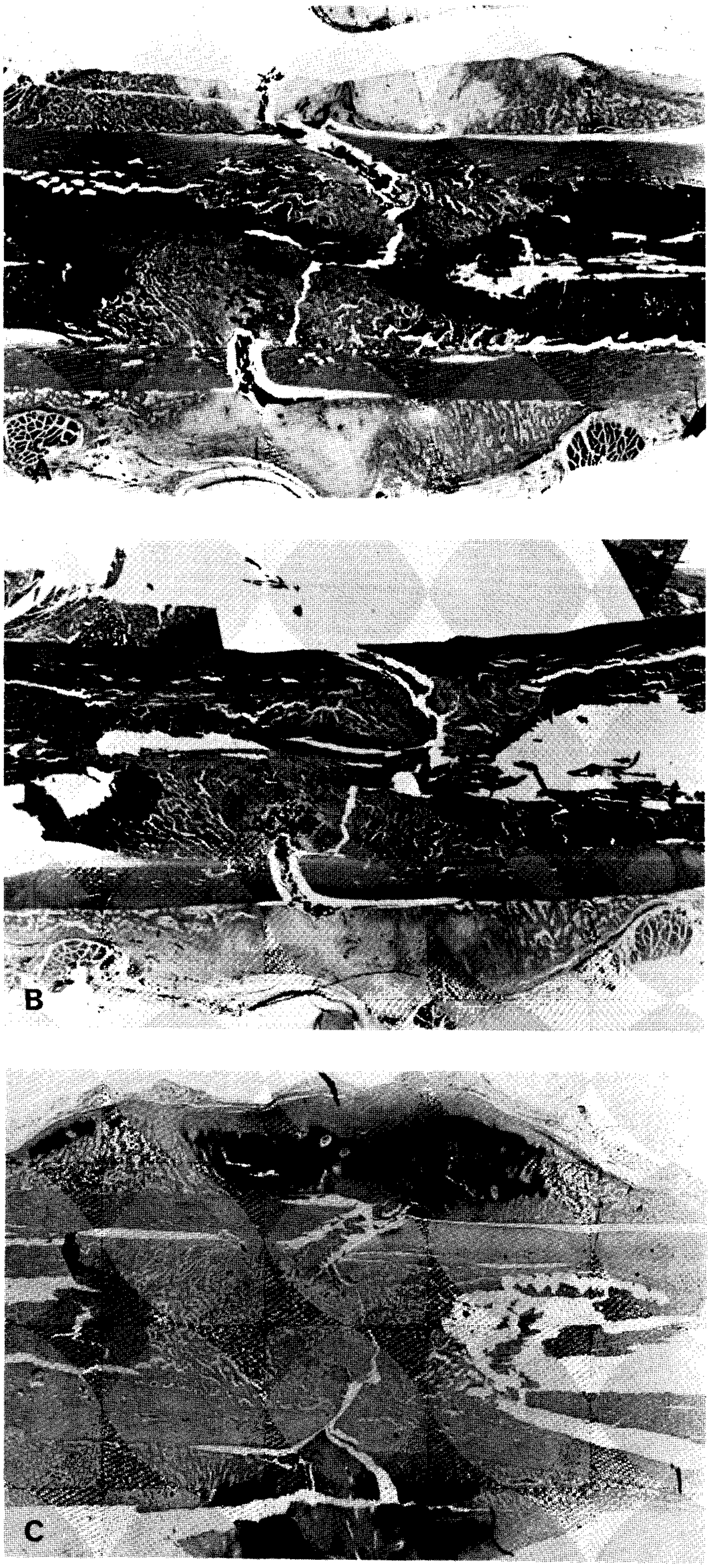


**Fig. 5A–C.** Histologic section (trichrome blue stain, × 1.25) of the ulnar fracture (**A**) which demonstrates the osseous and endosteal bridging also noted on the H and E ( × 1.25) stain (**B**).
The Alcian Blue stain emphasizes the cartilaginous component (**C**)

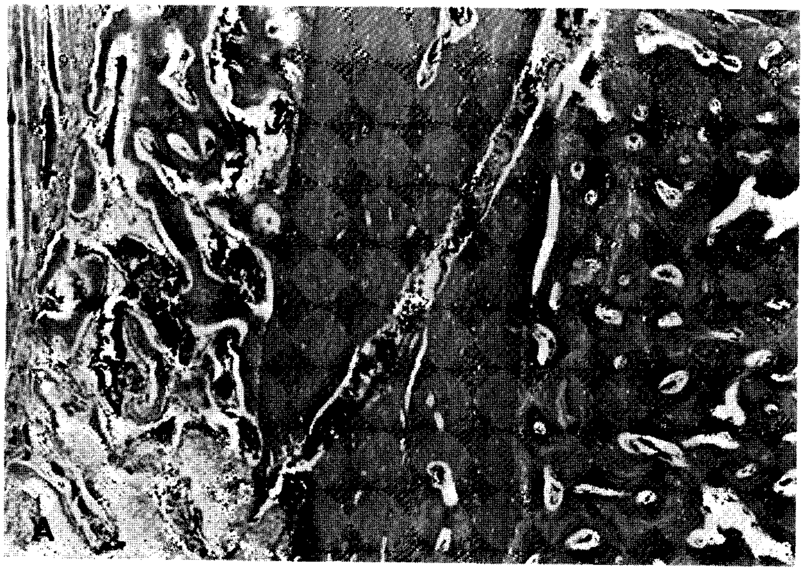

**Fig. 6. A** Xeroradiograph (negative mode) at 16 days demonstrating healing of the ulnar fracture. The humeral fracture remains displaced and no osseous bridging of that fracture is seen
**B** Histologic section (H and E, × 1.25) of humeral fracture at 16 days. Only minimal bony and cartilaginous callus has formed. Fibrous connective tissue is the major component of the healing process

## Discussion

The sequence of events leading to fracture healing in mammals has been described by a number of authors [14, 17, 20]. Hemorrhage and organization of clot is considered an integral part of the healing process [8, 9, 11]. Without hemorrhage and sufficient clot formation, fluid may fill the area and form fracture cartilage which is converted to bone by enchondral ossification [9].

Early in fracture healing vascular invasion from the periphery proceeds along precipitated guidelines, followed by proliferation of granulation tissue, woven bone production, and formation of trabeculated bone [4, 14, 20]. This sequence of events should occur in avian fractures of the radius and ulna but in the humerus, clot production may not lead to gel formation and fracture repair may occur by endochondral ossification. In avian species, replacement of bone marrow and vascular structures by the air sac diverticula has rendered proximal bones of the extremities relatively avascular [5, 6, 7, 12]. We believed this afforded a unique model to study the relative importance of hemorrhage in the fracture repair process and wished to test the value of xeroradiographic imaging in this analysis [2].

The general methodology of this study was to document the radiographic changes that occur with the two types of avian fracture healing and correlate them with histopathologic findings. We realized that many variables are inherent in this approach, which include the severity of induced fractures, mechanical



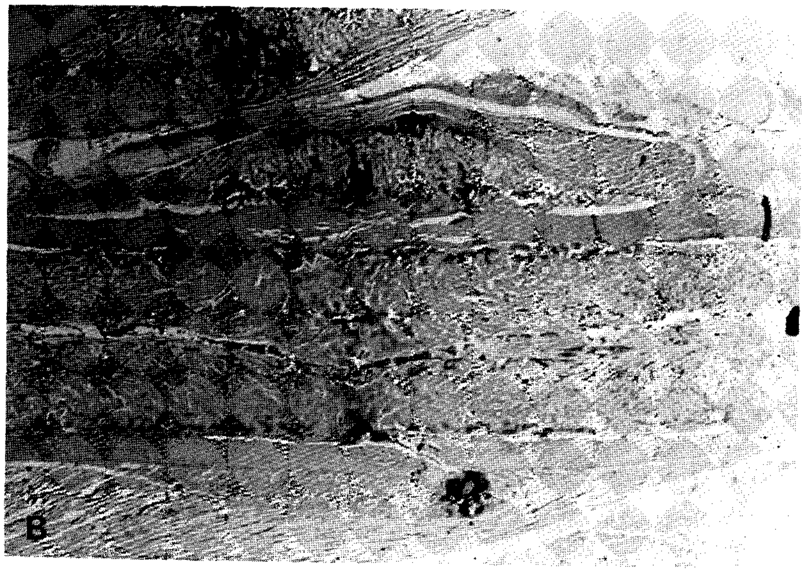


**Fig. 7A and B.** Histologic section of 16-day-old ulnar fracture (H and E, × 10). The healing is mainly cancellous bone with some cartilage formation of the periosteal side delineated by the Alcian Blue stain ( × 1.25)
[Adapted from Healing of Avian Fractures. Bush, Montali, Novak and James. J. Am. Anim. Hosp. Assoc. **16**, 768 (1976)]

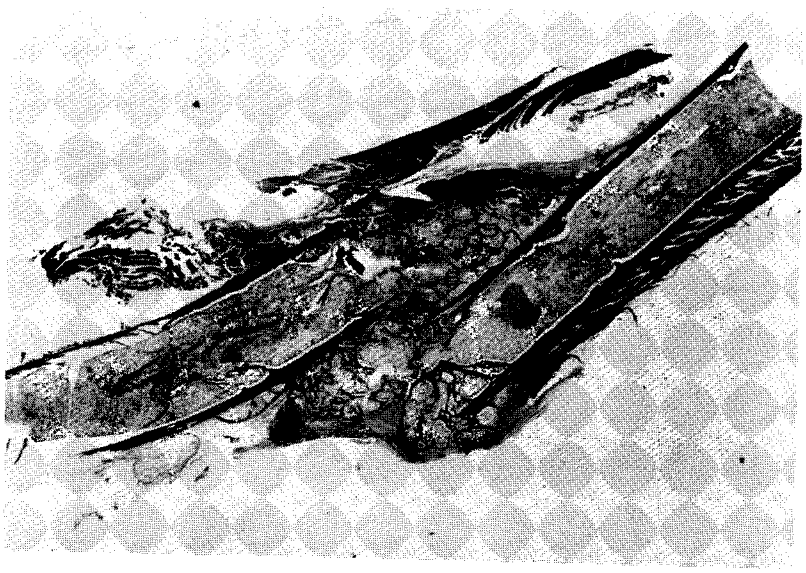


**Fig. 8.** Histologic section of humeral fracture at 16 weeks appears essentially unchanged from 16 days. (H and E stain, × 3.15)

displacement, and movement at the fracture site. All of these variables will alter the progression and rate of fracture healing, but represent the circumstances seen clinically. Our clinical treatment of the fractures attempted to minimize these factors.

The mechanism of fracture repair in avian species did not appear significantly different from that of mammals. Major callus formation appeared to be derived from blood clots associated with the fracture site. The emphysema that occurred initially around the fracture site was reabsorbed within 24 h which was well documented by the xeroradiographic images. These histologic studies indicate that connective tissue elements proliferate from the endosteal surface of the portions of the humerus lined with air sacs. Medullary bone fractures healed more rapidly than pneumatized bone which was demonstrated by xeroradiography. This may represent the time course of healing with and without adequate clot formation but may also be due to motion at the fracture site. Clinical treatment was designed to minimize wing motion during the healing phase [1, 16, 19].

In evaluating avian fractures, we found xeroradiographic evaluation quite helpful in visualizing callus formation. The correlation with the histologic changes was excellent. Signs of a bridging bony callus were observed on the xeroradiographic images at approximately two weeks in the radial and ulnar fractures and were clearly delineated by three weeks. Xeroradiography increased the diagnostic capability of fracture evaluation. The quality of edge enhancement allowed excellent delineation of associated changes in soft tissue density which has been previously described [15]. Comparing the appearance of the fracture site with the serial histologic findings, it appeared that secondary evidence of fracture repair could be demonstrated before actual callus formation was occurring.

In conclusion, there were significant temporal differences between the healing of fractures of pneumatized and medullary bone and xeroradiographic imaging provided an effective monitor to evaluate the sequence of events.

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