EPIPHYSEAL PLATE REPAIR USING FAT INTERPOSITION TO REVERSE PHYSEAL DEFORMITY

An experimental study

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by

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SUMMARY

A predictable method of reversal of physeal (epiphyseal plate) damage at the level of deformity is not currently available.

A reproducible model of a peripheral partial bone bridge to the proximal medial tibial physis was created in lambs at the age of 6 weeks. Three months after the initial procedure, reversal of the bone bridge lesion was achieved by excision of the bone bridge and autologous fat interposition from the ipsilateral knee fat pad.

There were 3 groups of animals with lesions to the physis. At reversal in the first group of animals, C.T. scans determined that the size of defects was 17.2 $\pm$ 2.2% of the total physeal area. For the second group of animals, the size of the defect was 28.0 $\pm$ 6.0%. For the third group of animals, the lesion was 33.7 $\pm$ 7.2%. Using an unpaired Student's *t* test, there was a statistically significant increase in size of lesion between groups ($p < 0.0001$).

With interposition of fat radiological criteria indicated success in the resumption of physeal growth in 85.7% of cases in the first group, 54.5% in the second group, and 50.0% in the third group.

The survival of the fat and its maintaining of position were critical factors to the success of the reversal procedures. For example, in the first series of experiments, success is 100% if two animals unable to satisfy both criteria are eliminated from analysis.

Measurements of the areas of the physeal defects using C.T. images between the initial (reversal) procedure and autopsy at three and six

months showed a decrease in size of the defects. These, however, failed to reach a statistically significant level of change in the size of the defects. This is presumptive evidence of the inability of physeal cartilage to repair.

Histological assessment showed that there was minimal evidence of physeal stimulation or resumption of a normal physis in the defects. A spur of cartilage could be seen to arise centrally from the physis and to point towards the metaphysis. In this region there was loss of normal physeal chondrocyte organization. Under polarized light microscopy the phenotypical expression showed metaplasia of the hyaline cartilage to fibrocartilage. Chondrons of physeal disorganization occurred.

It is postulated that correction of deformity occurs by a non-union effect, which allows the remaining normal physis to function and grow, rather than by a process of interstitial physeal repair.

In the first group of animals where the area of defect was 17.2 $\pm$ 2.2%, the defect may be resected with a 100% success rate. With larger defects, reversal of deformity may be partial or may not occur. Success was determined by the size of the lesion and the absence of bone bridge reformation.

The clinical significance of this study is that peripheral defects of < 17.2% of growth plate area will respond successfully to physeal interpositional surgery.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. I further consent to the thesis being made available for photocopying and loan as applicable, if accepted for the award of the degree.

BRUCE K. FOSTER

ACKNOWLEDGEMENTS

The facilities of the Flinders Medical Centre Animal House were made available with approval by the Flinders Medical Centre Ethics Committee. Mr. Ray Yates and staff (particularly Mrs. Christine Hill) were always prepared to put in an extra effort to care for the animals. Mr. Yates and his family provided an animal husbandry system to obtain lambs of known age. Through the Institute of Medical and Veterinary Science Animal House, Mrs. Alma Bennett and Mrs. Anne Schloithe gave additional animal care facilities as logistics sometimes required. Other personnel who assisted at operations were Mr. Phil Hutchins, and Mrs. Alana Hansen. Mrs. Hansen also gave exceptional additional technical assistance.

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Computerized planimetry of the C.T. images after selection of the most appropriate cut was undertaken independently by Mr. Winston Francis (Radiotherapy Department, Royal Adelaide Hospital.) Dr. Nick Fazzalari (Institute of Medical and Veterinary Science) and Dr. Ole Wiebkin (Department of Medicine, Royal Adelaide Hospital, University of Adelaide) gave statistical advice.

Dr. Michael Rozenbilds (Pathology Department, Flinders Medical Centre) supervised the production of the histopathological specimens and together with the author examined and re-examined specimens using

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DEDICATED TO

Penny and my family for providing an emotionally supportive environment.

Chapter 1 GLOSSARY

ARTHROTOMY: Opening of a joint by an incision.

DIAPHYSIS: The main shaft of a bone, between the two physes which mineralizes from a primary ossification centre.

EPIPHYSIODESIS/ PHYSIODESIS: Creation of a partial or complete bone bridge to join across the physis between the epiphysis and metaphysis.

EPIPHYSIS: The juxta-articular bone, ossified from a separate ossification centre.

INTERPOSITION: Placement of material between the epiphysis and the metaphysis at the level of the physis.

METAPHYSIS: The region of the diaphysis of tubular bones immediately near to the physis, where mineralization of the growing cartilage takes place.

PHYSIOLYSIS: To release or dissolve an abnormal bone bridge across the growth plate.

PHYSIS/ EPIPHYSEAL PLATE/ GROWTH PLATE: The disc of specialized hyaline cartilage that lies at the ends of the tubular bones providing for longitudinal bone growth.

Chapter 2 AIMS

The physis (epiphyseal plate) lies at the end of the long bones and during growth is responsible for bone lengthening.

The complication of limb shortening and/or angulation due to premature growth arrest of the physis presents a challenge to orthopaedic surgeons. Such arrest of bone growth may be partial or complete, central or peripheral, and give a variable degree of deformity as a consequence.

The hypothesis is that the physis has an internal mechanism of repair to restore physeal function.

The aims of this experimental study were to establish a defined degree of deformity by partial growth plate excision, and then to examine different methods of reversal of such deformity to observe the process of growth plate repair. The secondary aim was to define the percentage of physis that could be resected yet still enable reversal of deformity.

Chapter 3 NORMAL PHYSEAL FUNCTION

3.1 INTRODUCTION:

There are many causes of physeal deformity.

Congenital absence may be partial as in Madelung's (1878) deformity of the radius, Blount's (1937) disease of the tibia, and Kirner's (1927) deformity of the terminal phalanx of the little finger or it may be complete, as in Proximal Focal Femoral Deficiency [Reiner 1901, Shands & MacEwen 1962] or Fibula Hemimelia [Thompson, Straub & Arnold 1957, Westin, Sakai & Wood 1976]. In addition, there are ill-defined inborn errors of metabolism such as achondroplasia [Parrot 1878] and the mucopolysaccharidoses that cause dwarfism [Hunter 1916-1917, Hurler 1920, Morquio 1929, Lamy & Maroteaux 1957, Scheie, Hambrick & Barness 1962, Sanfilippo, Posodin, Langer & Good 1963].

There are many acquired aetiologies. The commonest cause of physeal deformity is injury [Hutchinsen 1894, Bowen 1915, Bergenfeldt 1933, Eliason & Ferguson 1934, Bisgard 1935, 1937, Ruckensteiner 1947, Harsha 1957, Budig 1958, Cassidy 1958, Dale & Harris 1958, Harris 1958, Brasheer 1958, 1959, Sakadida 1964, Aitken 1965, Steinert 1965, O'Brien, Morgan & Suter 1971, Sussenbach 1972, Tachdjian 1972, Rang 1974, Ogden 1978, Bouyala & Rigault 1979, Lovell & Winter 1986, Mizuta, Benson, Foster, Paterson & Morris 1987].

A clinical example of a physeal injury producing deformity is illustrated in the case of K.M. an 8 year old female who sustained a distal femoral fracture. 12 months after injury she developed a 10° valgus deformity of the distal femur and knee compared to the normal 3° of valgus. There was 2.1 cm. of shortening (Fig. 3.1).

Infection, either following a compound fracture or as a result of septic arthritis or osteomyelitis, may also cause physeal damage [Duthie & Ferguson 1973, Letts 1988].

Vascular occlusion causes physeal damage. This may result from:

(i) frostbite [Straub 1929, Löhr 1930, Bennett & Blount 1935, Greene 1943, Crismon & Fuhrman 1947, Thelander 1950, Shumacker & Lempke 1951, Pirozynski & Webster 1953, 1954, Blaustein & Siegler 1954, Dreyfuss & Glimcher 1955, Bigelow & Ritchie 1963, Morscher 1967].

(ii) burns [Evans 1959, Frantz 1966].

(iii) irradiation damage [Stevens 1935, Judy 1941, Spangler 1941, Barr 1943, Langenskiöld 1953, Baserga 1961, Barnhand 1962, Argüelles 1977].

(iv) electrical injuries [Kolar & Vrabec 1960, Brinn & Moseley 1966, Ogden 1981].

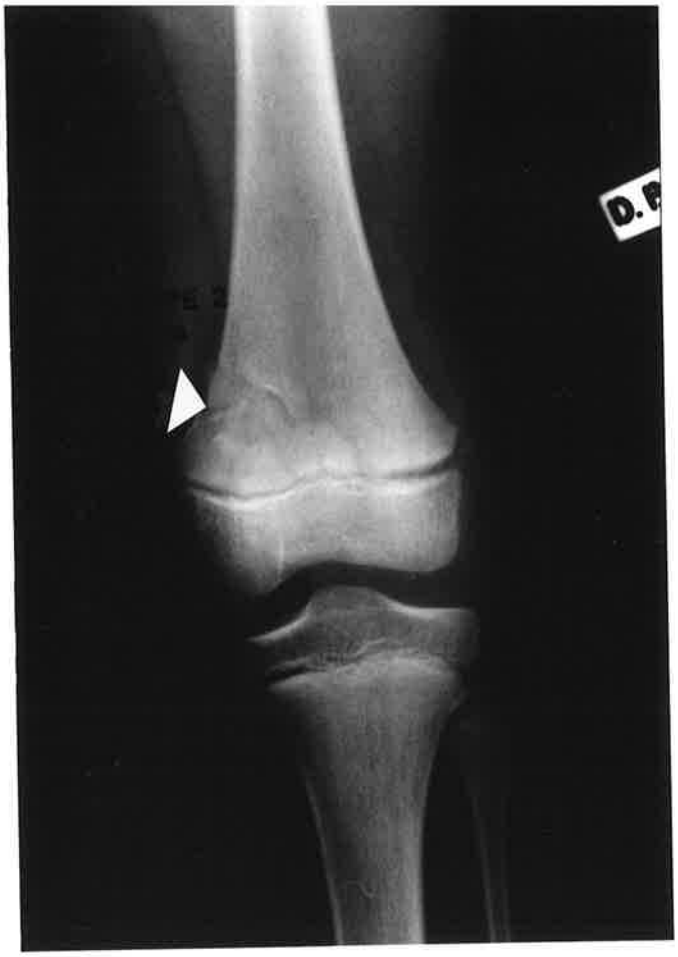
Vascular occlusion may also be a result of treatment, as in the treatment of slipped upper femoral capital epiphysis [Waldenström 1940, Ponseti & McCintock 1956] or the treatment of congenital

Figure 3.1: GROWTH ARREST FOLLOWING TRAUMA.

Figure 3.1.1: AP and lateral x-rays show a relatively undisplaced Salter-Harris type 2 fracture of the distal femoral physis in an 8 y.o. female. The marker shows the metaphyseal fragment and fracture line.

Figure 3.1.2: Lateral growth arrest 12 months later. On the AP and lateral x-rays the bone bridge is shown.

The C.T. scanogram shows a leg length discrepancy of 2.1 c.m. and the valgus deformity of the leg.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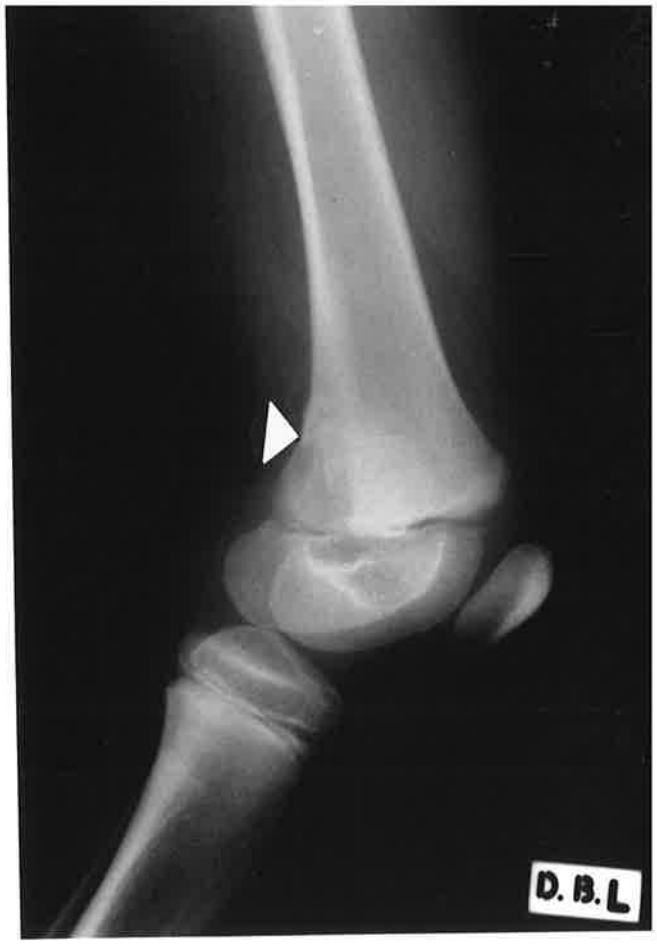
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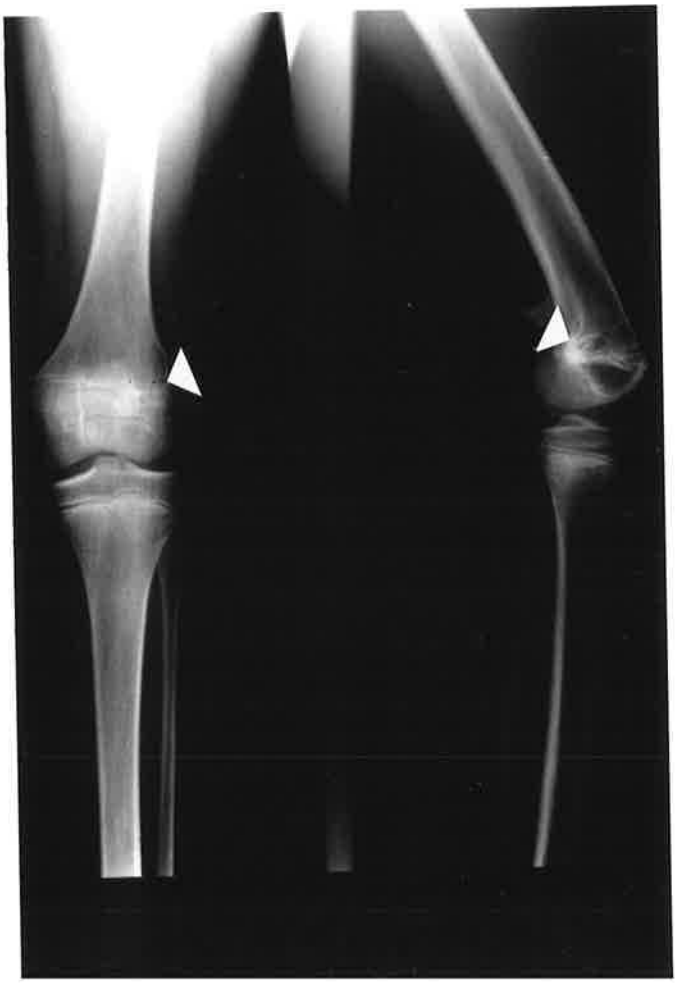

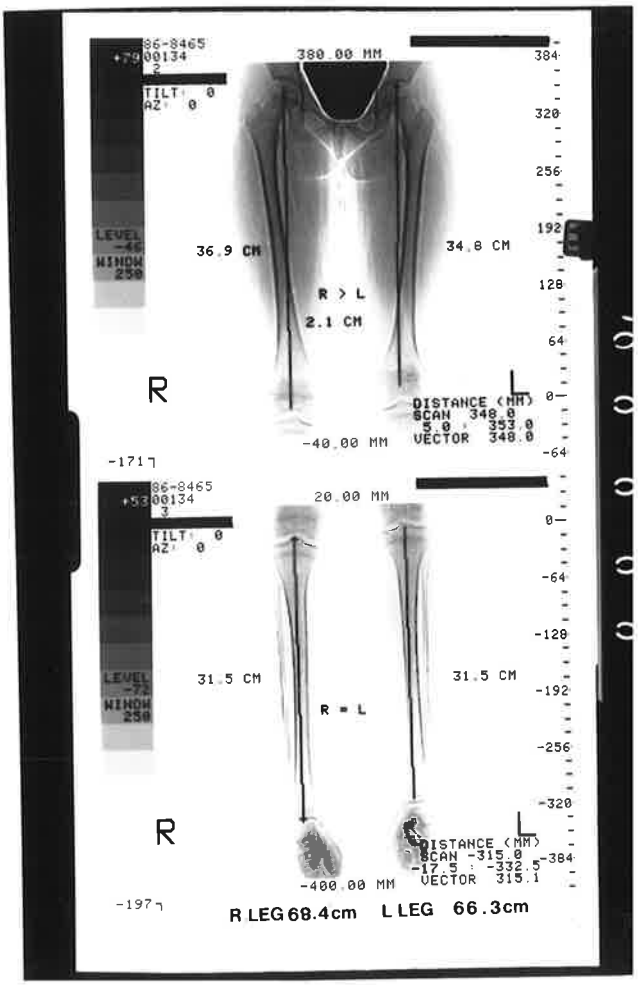
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380.00 MM
TILT: 0
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LEVEL
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31.5 CM
R = L
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VECTOR 315.1
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-192
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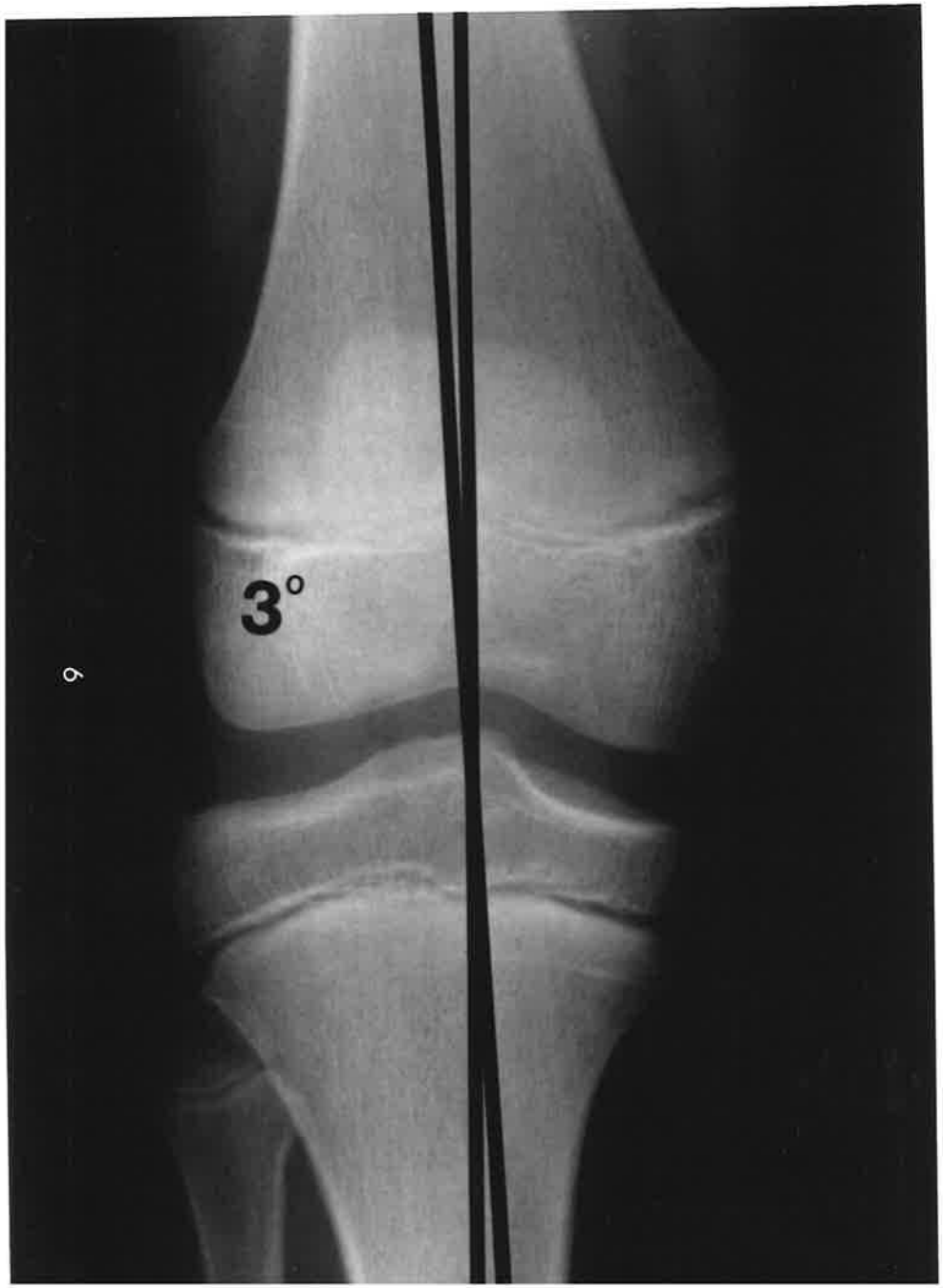
R LEG 68.4cm L LEG 66.3cm

Figure 3.1.3: AP x-ray shows the increased valgus angulation of 10° compared to the normal valgus of 3°.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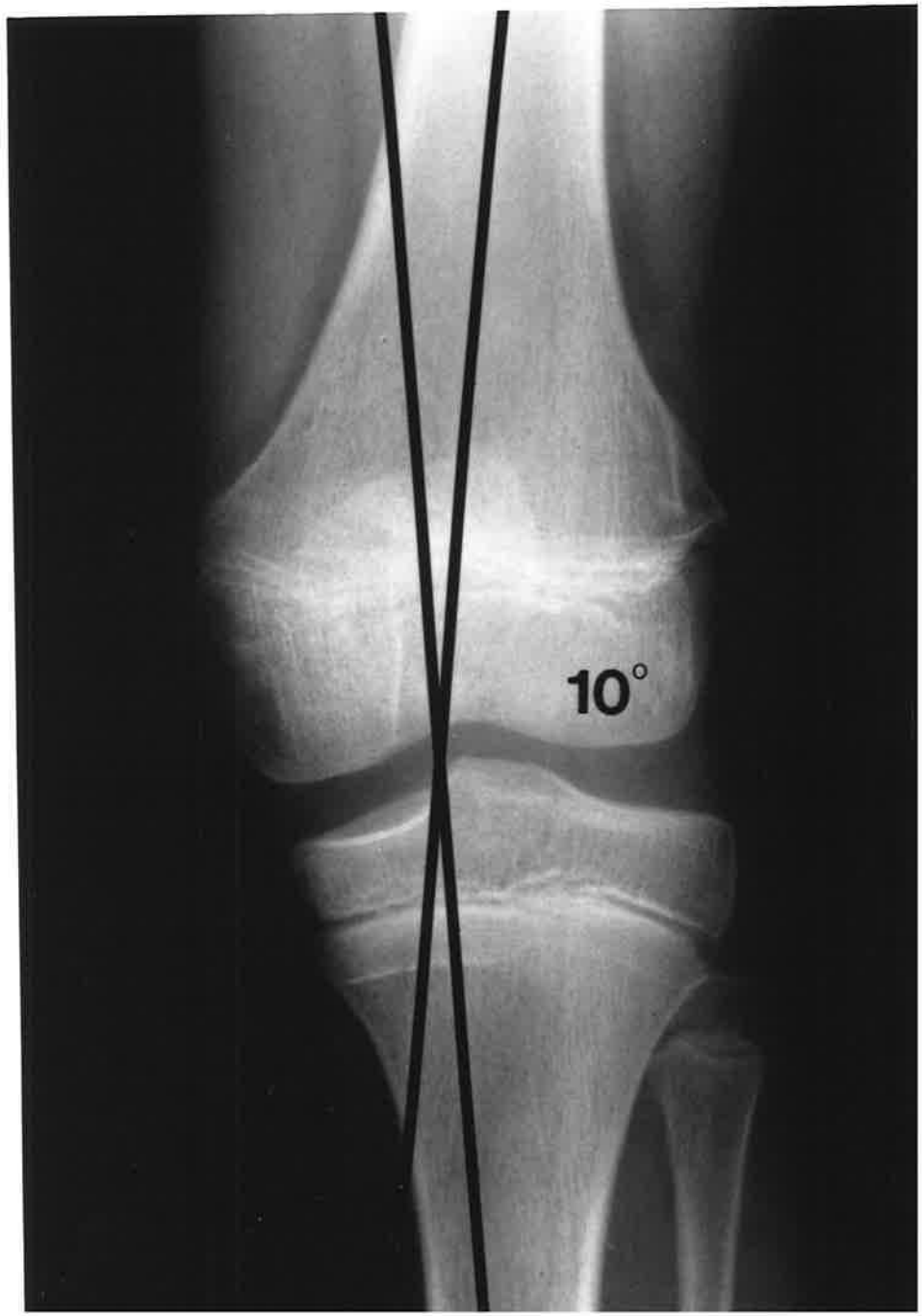

10°

dislocation of the hip [Salter, Kostuik & Dallas 1969, Bucholz & Ogden 1978, Kalamchi 1978, Kalamchi & MacEwen 1980, Cooperman 1980].

The author's own interest in physeal damage was stimulated in 1980/81 when, as a Research Fellow at the Alfred I. duPont Institute in Wilmington, Delaware, U.S.A., a review of patients with Perthes Disease was undertaken [Bowen, Schreiber, Foster & Wein 1982, Bowen, Foster & Hartzell 1984]. The review showed a significant incidence of physeal involvement as well as epiphyseal avascularity. This was prognostically important since the degree of femoral head deformity and probability of long-term hip degenerative arthritis correlated with the degree of physeal damage.

This thesis is concerned with the reversal of a mechanically induced lesion to the peripheral zone of the physis.

3.2 MECHANICAL PHYSEAL INJURY:

Of all mechanical injuries to the long bones during childhood approximately 15% involve the physis [Bowen 1915, Bergenfeldt 1933, Eliason & Ferguson 1934, Bisgard & Martenson 1937, Neer & Horwitz 1965, Salter 1970, Peterson & Peterson 1972, Tachdjian 1972, Rang 1974, Ogden 1982*a*, Lovell & Winter 1986, Mizuta et al 1987].

The relative incidence of specific areas of physeal injury is shown in Table 1 [Neer & Horwitz 1965, Peterson & Peterson 1972, Ogden 1982*a*, Mizuta et al 1987].

TABLE 1 Relative Incidence of Physeal Injuries

PHYSEAL SITE	Neer & Horwitz		Peterson & Peterson		Ogden		Mizuta Benson & Foster et. al.	
	No.	%	No.	%	No.	%	No.	%
Proximal clavicle	-	-	-	-	3	0.9	0	0
Distal clavicle	-	-	-	-	1	0.3	3*	0.9
Proximal humerus	72	3.0	22	6.7	27	6.1	7	2.0
Distal humerus (including epicondyles)	332	14.0	20	6.1	56	12.6	24	6.9
Proximal radius	124	5.2	1	0.1	5	1.2	16	4.6
Proximal ulna	21	0.9	-	-	3	0.9	1	0.4
Distal radius	1096	46.2	98	29.8	114	25.7	100	28.4
Distal ulna	136	5.7	12	3.7	11	2.5	16	4.6
Metacarpals	-	-	10	3.0	8	1.8	15	4.3
Phalanges (fingers)	-	-	39	11.8	41	9.3	91	25.8
Pelvis	-	-	-	-	23	5.2	0	0
Proximal femur	-	-	7	2.2	9	2.3	0	0
Trochanters	-	-	-	-	4	1.0	0	0
Distal femur	28	1.1	18	5.5	17	3.8	1	0.4
Proximal tibia	17	0.7	6	1.8	2	0.6	4	1.2
Proximal fibula	2	0.2	-	-	2	0.6	0	0
Tibial tuberosity	-	-	-	-	12	2.7	0	0
Distal tibia	238	10.2	59	17.9	60	13.5	33	9.3
Distal fibula	302	12.8	21	6.4	15	3.4	12	3.5
Metatarsals	-	-	6	1.8	3	0.9	5	1.5
Phalanges (toes)	-	-	11	3.7	21	4.7	25	6.2
	2368	100%	330	100%	443	100%	353	100%

In 1965 a review by Neer and Horwitz covered 2,368 consecutive physeal injuries. This and other reviews showed that males sustain physeal injuries more frequently than females. The ratio is variably reported as ranging from 4:1 to 9:1. It has been proposed in the literature that the significant aetiological factor here is the greater exposure of boys to trauma from athletic activities. Additionally the physes of the male also stay open longer than the physes of the female, extending the period of possible trauma. The study by Mizuta, Benson, and Foster et al, in 1987 confirmed these findings, and suggested that the site of injury was an important prognostic factor. It also confirmed that for a hospital of primary referral (The Adelaide Children's Hospital) the incidence of physeal injury was 17.9%.

There may also be different responses of the physes to trauma during the adolescent growth spurt. Morscher et al (1965, 1968) has shown that there are hormonally mediated differences in the physeal response to experimentally applied stresses.

All studies have shown that in all the long bones the distal physes are injured more commonly than the proximal physes (Table 1). This higher incidence of injury to the distal radius, distal tibia and phalanges may result from the increased exposure of these more distal regions to trauma rather than from any unique physiological susceptibility of these particular physes.

These studies also show that there are 2 age periods of rapid skeletal growth: the first year of life, and adolescence (9-12 in girls; 12-13 in boys). Fractures involving the physes commonly occur

during these periods. This suggests that at the same physiological age the physes of males and females are equally susceptible to injury.

3.3 CLASSIFICATION OF FRACTURES:

In 1863 Foucher first proposed a classification of physeal injury. It recognised that the level of the line of separation between the physis and metaphysis is consistent. This acknowledged the recognition in the early 19th century that this was a different type of fracture to that of the diaphysis.

In 1894 Hutchinson wrote in the English literature of an increasing awareness of these injuries. In 1898, Poland advanced a classification of 4 types of fractures, based on a review of museum specimens and experimental cadaver work (Fig. 3.2).

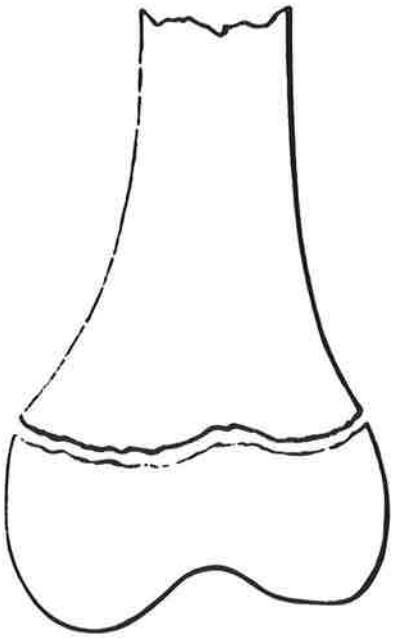
Aitken (1965) presented a classification which designated 3 types of fractures. He was the first to emphasize that deformity as a result of initial malunion or potential growth disturbance was rare, although the degree of initial displacement was significant. Aitken however, emphasized that the compression injury which was extremely difficult to diagnose, and was generally thought to be of little significance, yet could easily lead to major growth deformities.

A detailed classification of fracture types involving the physis was proposed by Salter and Harris in 1963. This classification was based on a combination of the mechanism of injury, relationship of the fracture line to the various cellular layers of the physis, and the prognosis following subsequent disturbance of growth.

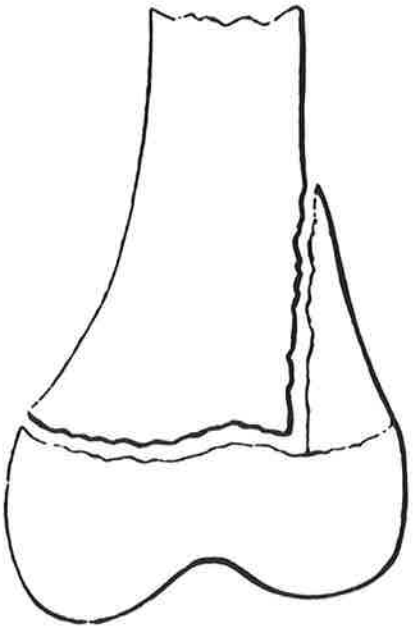
Figure 3.2: POLAND'S CLASSIFICATION OF PHYSEAL FRACTURE.

There are 4 types of injury.

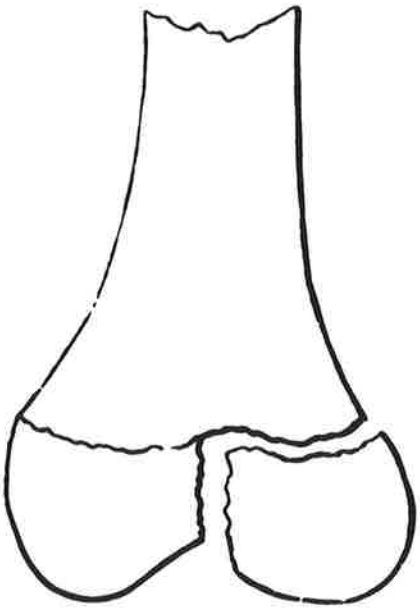
(Reproduced with permission from Poland, J.: Traumatic separation of the epiphysis. London, Smith, Elder and Co., 1898 by courtesy Fractures in Children, Rockwood et al, J.B.Lippincott, 3: 1984.)



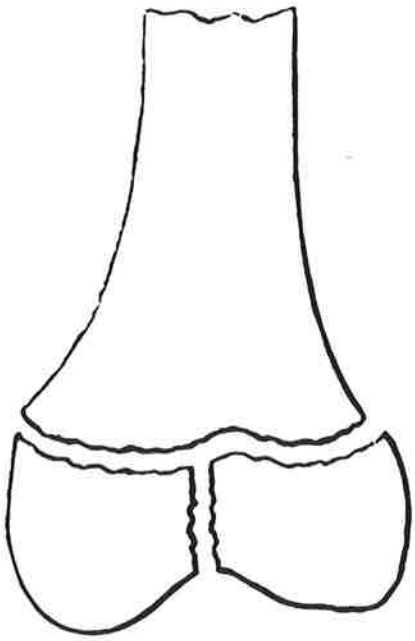
Pure and complete separation



Partial separation, with fracture of the diaphysis



Partial separation, with fracture of the epiphysis



Complete separation, with fracture of the epiphysis

Salter and Harris recognised five radiologically definable groups (Fig. 3.3).

Type I: There is complete separation of the epiphysis from the metaphysis without any osseous fracture. The growing cells of the physis remain with the epiphysis.

This type of injury is common in early childhood when the physis is thick. It is also seen in pathological conditions with physeal separation, for example in scurvy, rickets, osteomyelitis and endocrine imbalance [Salter 1970]. This last factor is thought to be significant in slipped upper femoral capital epiphysis [Harris 1950].

Wide displacement is uncommon because the periosteal attachment remains intact. Reduction is not difficult, and the prognosis concerning future growth is excellent unless the epiphysis is intra-articular [Dale & Harris 1958], in which case the blood supply may be damaged resulting in premature closure of the physis.

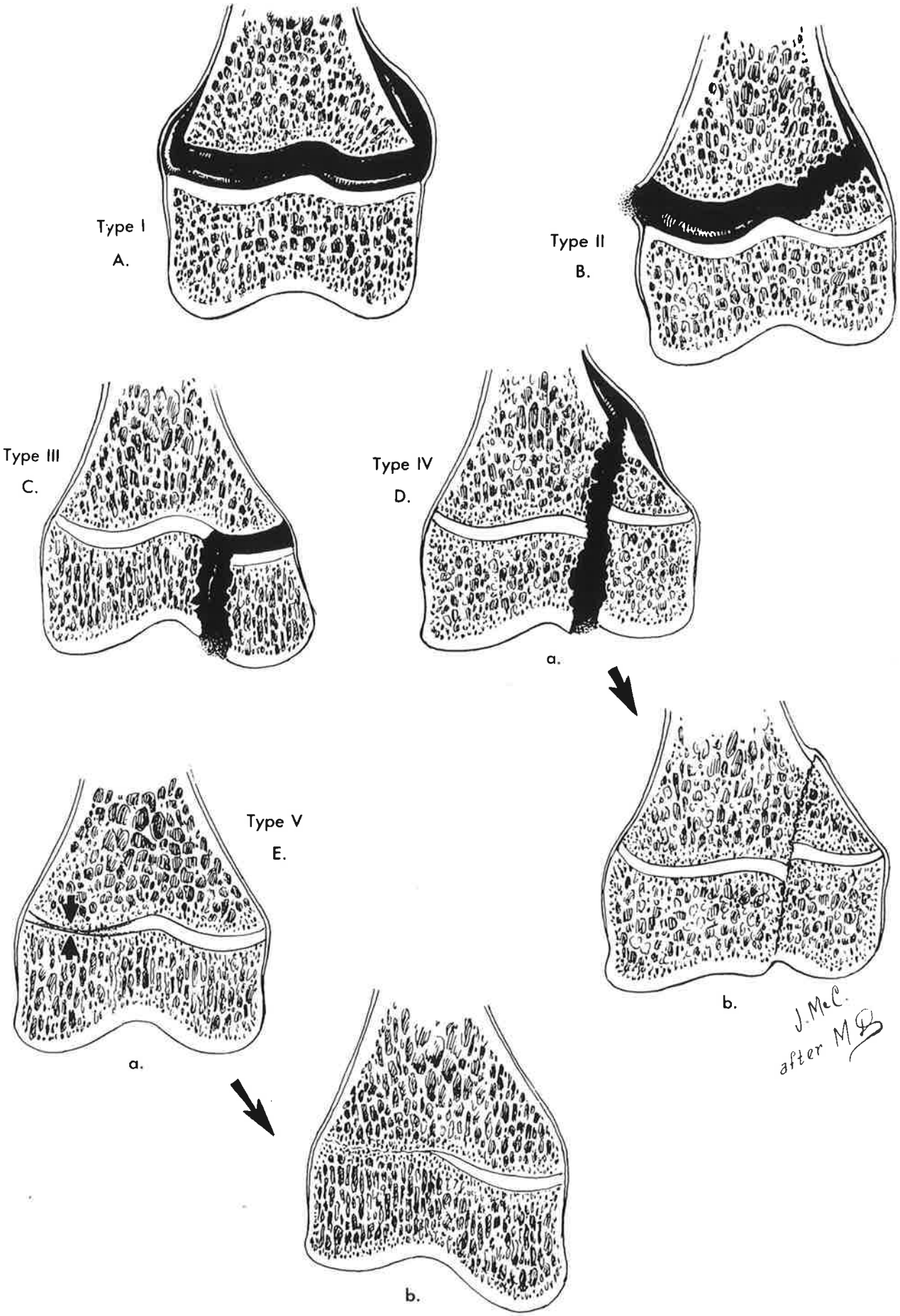
Type II: The injury occurs where the line of separation extends along the physis to a variable distance and then out through a triangle of metaphyseal bone. This is called Thurston Holland's sign (1929). It is the commonest type of fracture.

This type usually occurs in children over 10 years of age. The periosteum is torn on the side opposite to the angulation, but is intact on the concave side.

The growing cartilage cells of the physis remain with the epiphysis.

Figure 3.3: SALTER AND HARRIS' CLASSIFICATION OF PHYSEAL FRACTURE.

(Reproduced with permission from Salter, R. B. and Harris, W. R. Injuries involving the epiphyseal plate. J. Bone Joint Surg., 45A: 587, 1963.)



Classification of epiphyseal plate injuries according to Salter and Harris.

The prognosis concerning growth is excellent, providing the circulation to the epiphysis remains intact, which is usually the case.

Type III: The fracture, which is intra-articular, extends from the joint surface to the level of the hypertrophic cells of the physis, and then extends along the growth plate to its periphery.

This type of injury is uncommon. The commonest sites are either the upper or lower tibial physis, and injury is due to intra-articular shearing forces.

Accurate reduction is essential to prevent bar formation across the physis and to restore a smooth joint surface.

As with Type I and II injuries, provided the blood supply to the separated epiphysis is intact the prognosis is good with accurate reduction.

Type IV: The fracture, which is intra-articular, extends from the joint surface through the epiphysis across the full thickness of the physis and through a portion of the metaphysis producing a complete discontinuity of epiphysis, physis and metaphysis.

Perfect reduction of a Type IV epiphyseal plate injury is essential for function of the epiphyseal plate, and for the restoration of a smooth joint surface [Salter & Harris, 1963].

Unless the fracture is undisplaced, open reduction is always necessary. The physis must be accurately realigned to prevent bony

union across the plate with resultant local premature cessation of growth. These fractures occur commonly at the lateral condyle of the humerus and the distal femoral physis.

Type V: This relatively uncommon type of injury results from a severe crushing force applied through the epiphysis to one area of the physis. It occurs in joints that normally move in one plane only, such as the ankle or knee. At the ankle a severe abduction or adduction injury to a foot which normally only flexes or extends is likely to produce crushing of the physis which may or may not separate. Displacement of the epiphysis under these circumstances is unusual, and the initial radiograph gives little indication of the serious nature of the injury. Indeed, the injury may be dismissed as a sprain. One must suspect compression of the physis under such circumstances, and hope to prevent premature cessation of growth by protection from weight bearing for three weeks [Salter 1970].

Thus, the prognosis for Type V physeal injury is poor and difficult to predict. A type V injury may occur in association with any of the other more defined types of injury.

Rang (1969) has added another peripheral physeal injury in which the Ranvier Zone is injured along the immediately adjacent metaphysis and epiphysis. It may arise as an extension of a traumatically induced infection or severe burn, or as a result of direct trauma to the plate, such as from a lawn mower injury or bike wheel spoke injury.

This has become known as an addition to the Salter-Harris group to be called a Type VI injury (Fig. 3.4).

Figure 3.4: RANG'S CLASSIFICATION OF A PHYSEAL FRACTURE. The type 6 injury importantly includes the peripheral physeal lesion.

(Reproduced with permission from Rang, M. The growth plate and its disorders. Baltimore, Williams & Wilkins, 1969.)

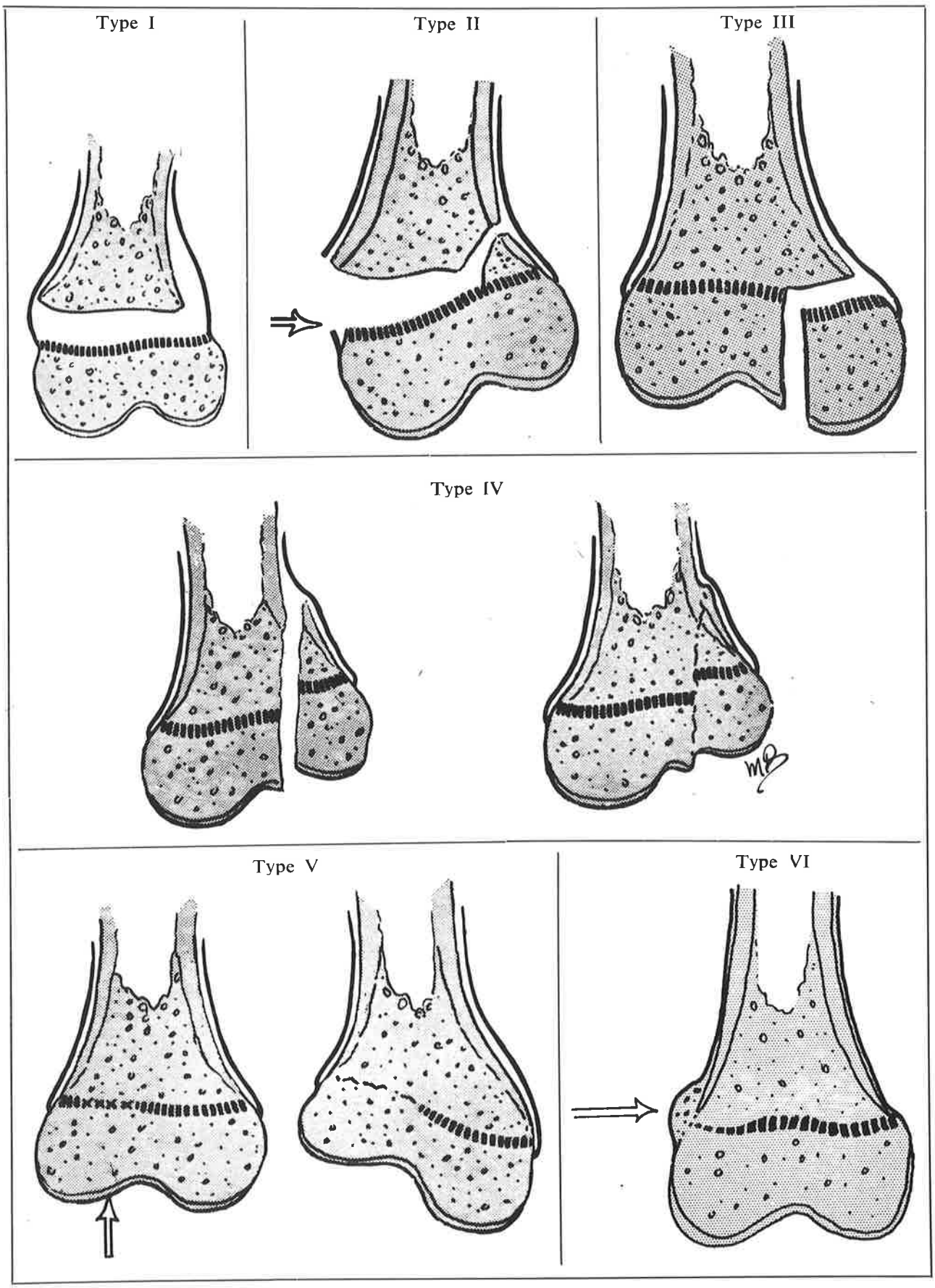

Type I
Type II
Type III
Type IV
Type V
Type VI

Figure 3.5: OGDEN'S CLASSIFICATION OF PHYSEAL INJURY.

1A: Propagation across the physeal cartilage.

1B: Propagation across the diseased primary spongiosa (e.g. leukemia, thalassemia) with the physeal interface variably involved.

1C: Disruption of a localized segment of the physis.

2A: Partial propagation across both the physis and metaphysis.

2B: Free and attached metaphyseal fragments.

2C: Propagation across both the primary spongiosa and metaphysis.

2D: Localized disruption of the physis at the point of propagation into metaphysis.

3A: Epiphyseal fragment with propagation through the physis.

3B: Epiphyseal fragment with propagation through the primary spongiosa.

3C: Crushing injury to peripheral physis.

3D: Nonarticular cartilage avulsion (e.g. ischial tuberosity).

4A: Combined epiphyseal-physeal-metaphyseal fragment.

4B: Epiphyseal-physeal-metaphyseal fragment combined with Type 3A or 3B lesion.

4C: Propagation through a nonarticular epiphyseal region (e.g.intraepiphyseal cartilage of the developing femoral neck.)

5: Longitudinal growth retardation of a major physeal segment.

6: Avulsion or crushing of the peripheral physis (zone of Ranvier).

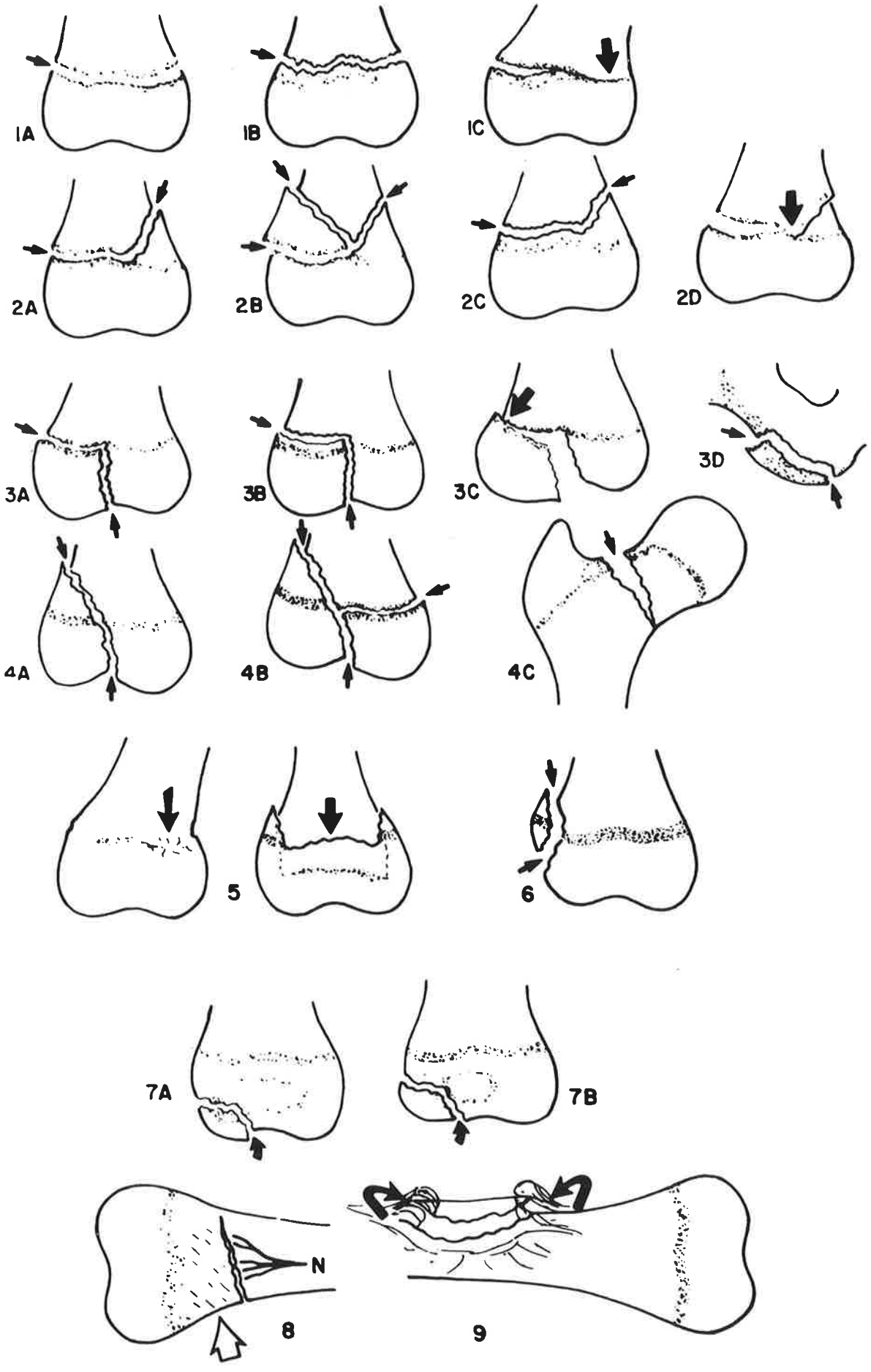
7A: Osteochondral fragment involving the physis of the secondary ossification center.

7B: Chondral fragment involving hypertrophic cells of the physis of the secondary ossification center.

8: A metaphyseal fracture temporarily cuts off the nutrient artery (N), causing transient ischemia to the metaphyseal segment between the fracture and the physis.

9: Damage to periosteum, with or without discrete osseous injury, disrupts normal membranous ossification.

(Reproduced with permission from Ogden, J.: J. Pediatr. Orthop., 2: 1982.)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7B
N
8
9

Certain types of epiphyseal and physeal injuries cannot be readily classified by the Salter-Harris classification, and complicated combination injuries occur. Furthermore, other growth mechanisms such as the zone of Ranvier, epiphyseal perichondrium and periosteum are not included.

Ogden (1982*a*, 1982*b*), proposed an enlarged classification to cover the other growth mechanisms. This classification is more complex and has yet to stand the test of time (Fig. 3.5).

3.4. ANATOMY & PHYSIOLOGY:

The physis is a complex structure with a high degree of cellular organization. It consists of hyaline cartilage cells which are anatomically and functionally different, but which merge into each other (Fig. 3.6, 3.7).

The immediately adjacent metaphysis is important as a functionally and anatomically totally independent structure. The metaphyseal blood supply contributes the vascular buds and pluripotential cells responsible for cartilage resportion and new bone formation.

The blood vessels to the physis must pass through a supporting bone plate to reach the cartilage basal cells. Immediately below the bony epiphysis one finds the zone of reserve or resting cells. These merge consecutively into the zone of proliferation, the zone of maturation or hypertrophic cells, the zone of calcification, and finally the metaphysis, where vascular invasion and bone formation occur (Fig. 3.6).

Figure 3.6: NORMAL PHYSEAL ANATOMY.

The morphological sequence of cellular organization is similar in all mammals.

Figure 3.6.1: The growth plate of the proximal epiphyseal plate in a rabbit's tibia.

A. Bone plate.

B. Zone of resting cells.

C. Zone of proliferation.

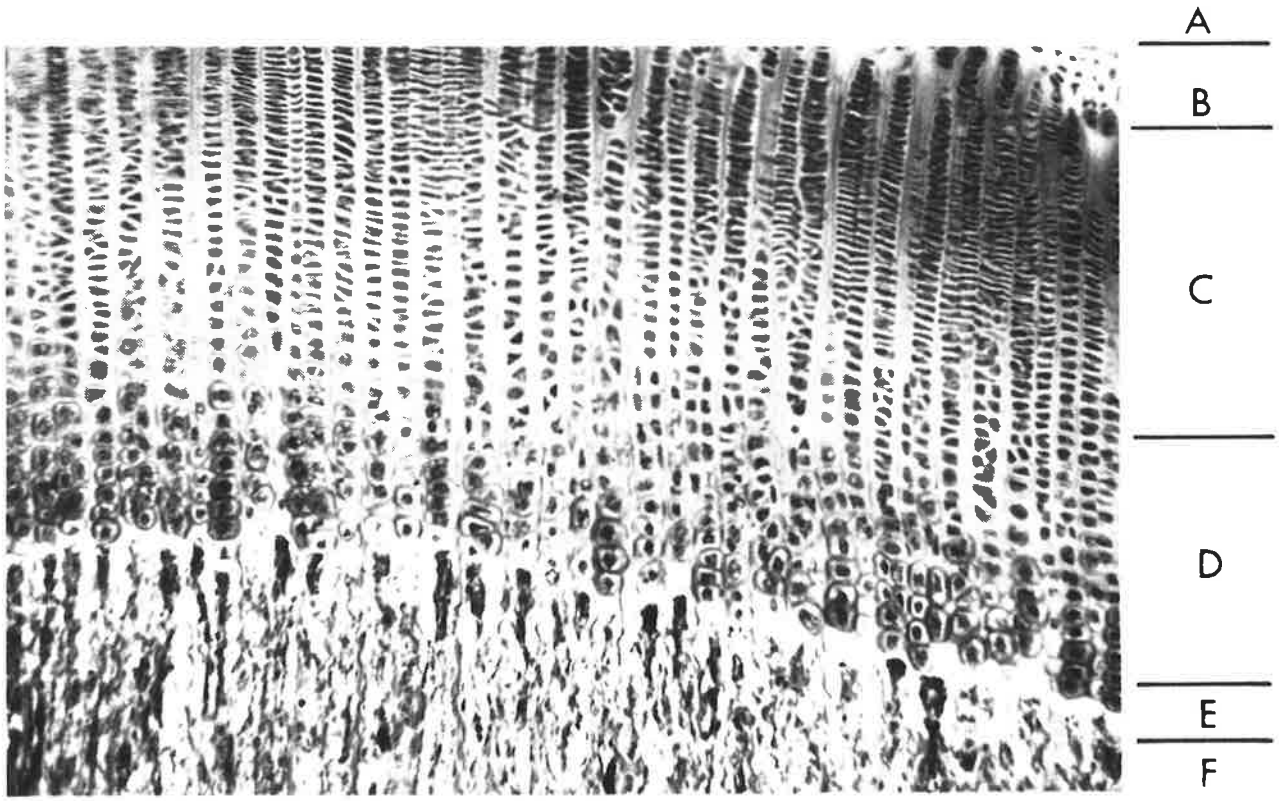
D. Zone of hypertrophic cells.

E. Zone of cell degeneration or provision calcification.

F. Layer of bone formation. (x 500)

(Reproduced with permission from Trueta and Morgan, 1960.)

Figure 3.6.2: Histological section of the proximal epiphyseal plate in a sheep's tibia aged 3 months. It shows the same structural organization of the specialized hyaline cartilage as the rabbit physis. (x 250)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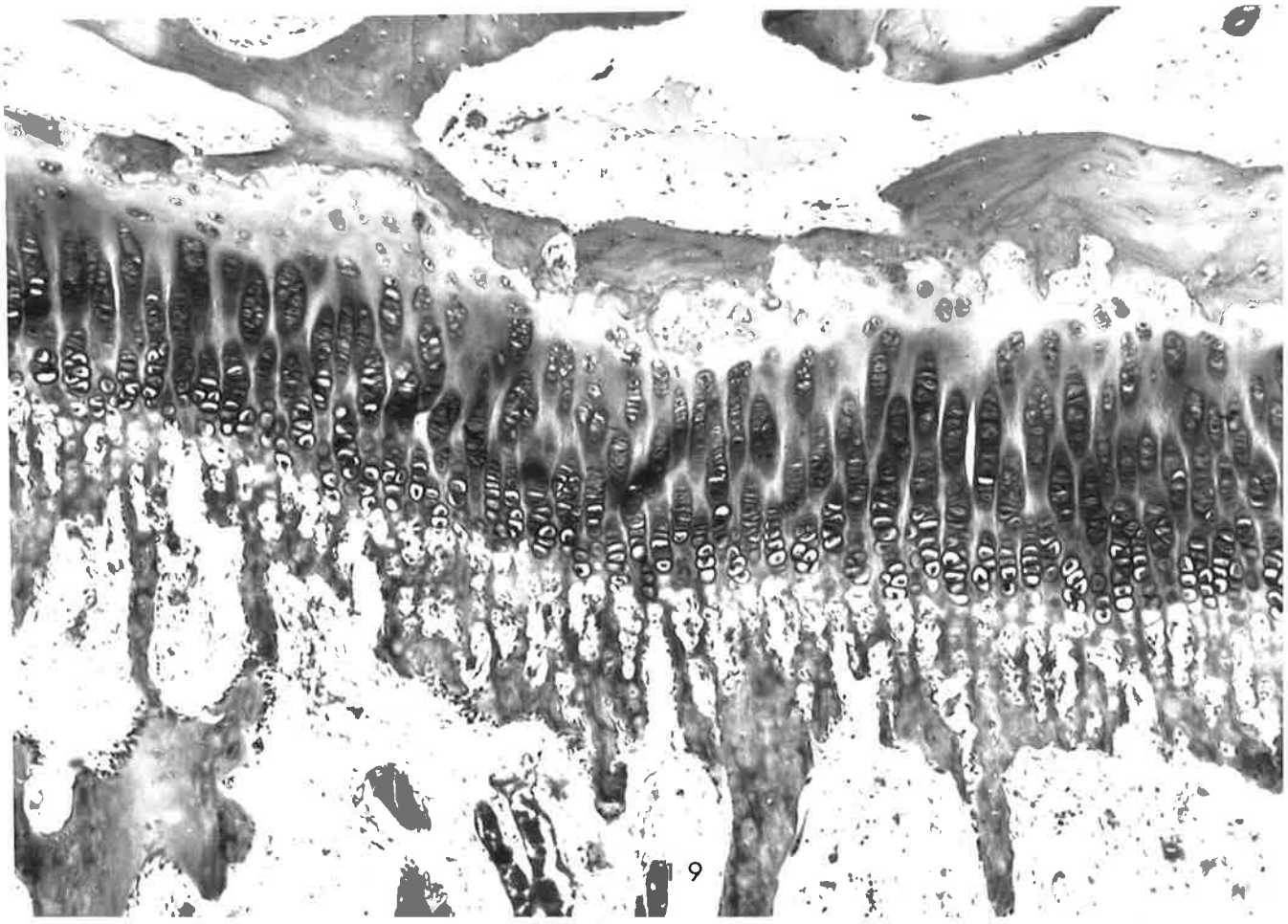
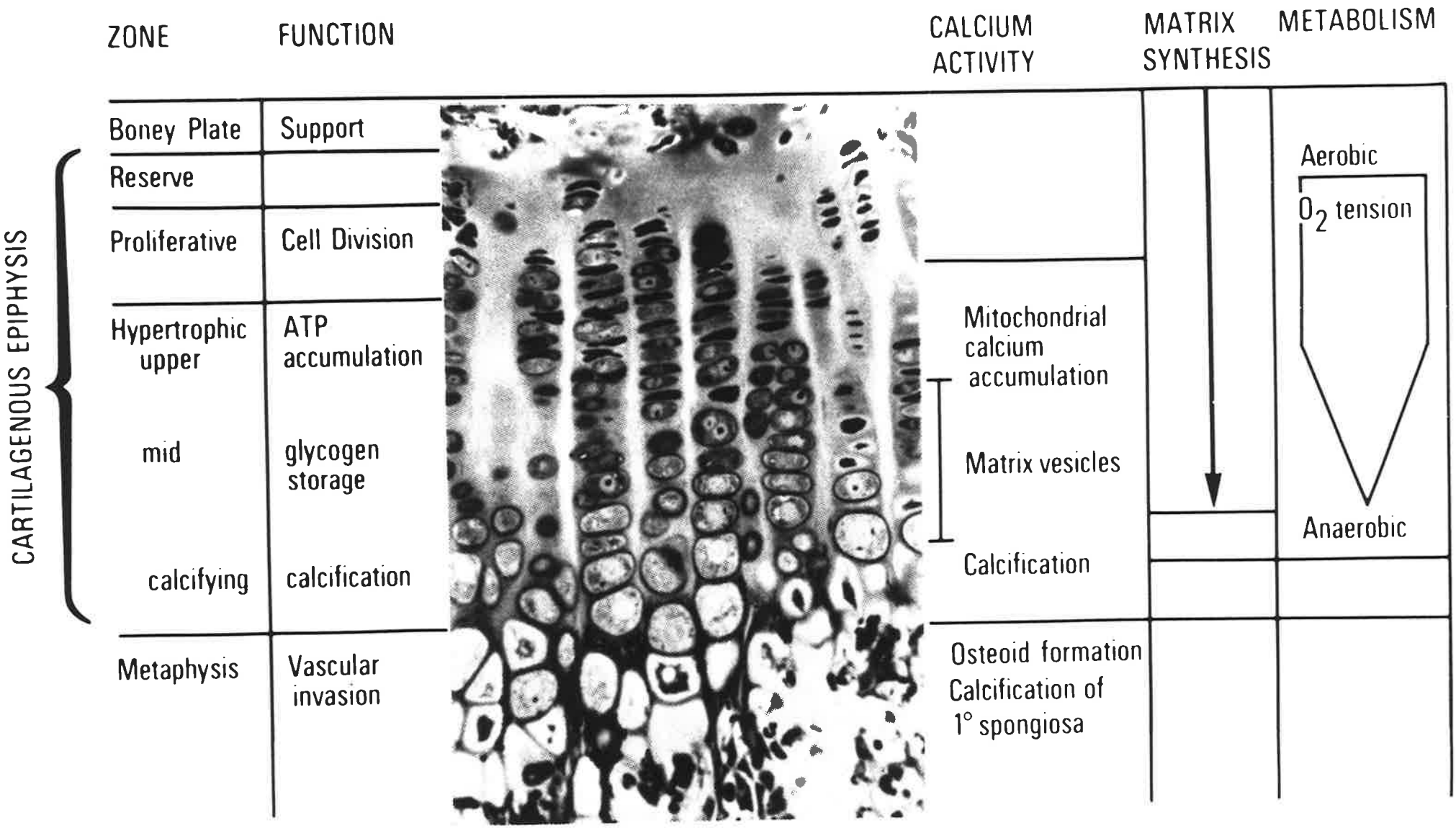


Figure 3.7: PHYSEAL ZONAL FUNCTION. Photomicrograph of the proximal tibial epiphysis of a rat showing the various zones of the epiphysis and their function.

(Reproduced with permission from Cruess, R. L. (ed.) The musculoskeletal system: embryology, biochemistry and physiology, New York, Churchill Livingstone, 193, 1982.)


ZONE
FUNCTION
CALCIUM ACTIVITY
MATRIX SYNTHESIS
METABOLISM
CARTILAGENOUS EPIPHYSIS
Boney Plate
Support
Reserve
Proliferative
Cell Division
Hypertrophic upper
ATP accumulation
mid
glycogen storage
calcifying
calcification
Metaphysis
Vascular invasion
Mitochondrial calcium accumulation
Matrix vesicles
Calcification
Osteoid formation
Calcification of 1° spongiosa
Aerobic
O_2 tension
Anaerobic

The function of the different zones of the physis can be summarized diagramatically (Fig. 3.7). The following describes the characteristics of each physeal zone.

3.4.1 Zone of Reserve Cells

The cells in the reserve zone are approximately the same size as those in the proliferating zone [Brighton, Sugioka & Hunt 1973, Brighton 1978, Hunziker 1987]. They are spherical in outline and exist alone or in pairs; they are not arranged in columns as are the cells of the other zones. Although named "resting cells" morphologically and biologically, the cells are not resting because they have abundant rough endoplasmic reticulum and are rich in lipid [Brighton, Sugioka & Hunt 1973]. However, Kuhlman in 1960, 1965 and Wray & Goodman in 1961, have demonstrated that their enzyme content as well as their content of ions, including calcium is the lowest of the entire physis. The intercellular matrix in this area is more abundant than in any other zone. It is higher in collagen content than those of other zones, and the collagen fibrils are randomly distributed [Irving & Wuthier 1968]. The proteoglycan, lipid, and water which constitute the other components of the intercellular matrix are present in smaller amounts than in the rest of the physis [Lindenbaum & Kuettner 1967]. Oxygen tension is low, [Anderson, Cecil & Sajdera 1975] indicating in all probability that the blood passing through the bony end plate to supply the reserve zone does not lose much of its oxygen to the cells. Autoradiographic techniques demonstrate that cells in this zone divide only occasionally [Kember 1960].

Brighton in 1978 has suggested that these cells store lipids and other nutrients for subsequent activity but this is not certain.

3.4.2 Zone of Proliferating Cells

The haphazard arrangement of chondrocytes of the reserve zone gradually line themselves up into vertical columns, with the long axes of the columns being parallel to the long axes of the bone.

The cells have a flattened appearance and there is a great increase in rough endoplasmic reticulum [Holtrop 1972*a*, Brighton, Sugioka & Hunt 1973]. Synthesis of proteoglycans in this region is the lowest in the entire physis [Greer, Janicke & Mankin 1968]. But ^{35}S incorporation has been demonstrated to be maximal in this zone [Schmidt, Rodegerdts & Buddecke 1978]. The intercellular matrix contains the highest content of proteoglycans, [Greer, Janicke & Mankin 1968] and therefore it is logical to propose that there is either a low rate of breakdown [Brighton 1978] or a real increase in proteoglycan synthesis with an increase in lysosoymal activity [Schmidt et al 1978].

The role of the proliferating zone is cell division, and it is in this layer that growth of long bones takes place. The proliferating cells are the only cells of the physis that normally divide, and growth in length is a direct result of that division. In 1960 Kember demonstrated by autoradiographic uptake of tritiated thymidine that the proliferating zone selectively takes up the nuclide.

Once cell division has occurred with each cell being lined up in a column, a change in intercellular activity occurs. The cell enters the hypertrophic zone as the cells more proximal to it, grow away from it [Hunziker 1987].

The oxygen tension in the zone of proliferation is the highest in the physis, which, when coupled with accumulation of glycogen in the cells, makes it likely that aerobic metabolism and storage of glycogen is occurring [Brighton 1978].

3.4.3 Zone of Hypertrophic Cells

From the proximal (epiphyseal) end to the distal (metaphyseal) end of the hypertrophic zone each cell diameter enlarges to five times its original size [Brighton, Sugioka & Hunt 1973, Buckwalter 1986, Hunziker, Schenk & Cruz-Orive 1987]. The large quantities of glycogen of the proliferative zone disappear in the middle of the hypertrophic zone [Pritchard 1952, Brighton, Ray, Soble & Kuettner 1969].

Morphologically, the cells that initially have well-formed ultrastructural elements gradually fill with holes that take up more than half the volume of the cell [Holtrop 1972*b*, Brighton, Sugioka & Hunt 1973]. At the metaphyseal end fragmentation and loss of subcellular detail occurs.

The oxygen tension is extremely low as a result of the increased distance from the epiphyseal blood supply, and Shepard and Mitchell in 1976 proposed that this relative anoxia may be responsible for the orderly sequence of cell activity.

The zone is rich in enzymes of the Krebs Cycle [Kuhlman 1960, 1965] and in lipids [Irving & Wuthier 1968]. The matrix contains the lowest content of both collagen and proteoglycan [Greer, Janicke & Mankin 1968, Irving & Wuthier 1968].

Electron microscopy has shown dense granules in the mitochondria of cells in this zone. Biochemical and histochemical evidence [Martin & Matthews 1970, Brighton & Hunt 1976] and x-ray diffraction studies have shown these to be calcium and phosphorous [Sutfin, Holtrop & Ogilvie 1971]. The mitochondria are most abundant at the epiphyseal end of the hypertrophic zone, but in the midportion they rapidly lose their calcium ions, [Brighton & Hunt 1976] and by the metaphyseal end both the mitochondria and cell membrane show no evidence of calcium retention [Arsenis 1972].

As the mitochondria lose calcium, matrix vesicles begin to appear outside the cells. These have been suggested as the initial site for mineral deposition [Anderson 1969, 1973, Boskey 1979, Arsenault 1988].

3.4.4 Mineralization

The details of calcification of the intercellular matrix of the physis are obscure. Within the physis there is a progression from a mineral-free state to the point where the cells gradually load their cellular membranes with calcium and then with calcium phosphate [Anderson, Cecil & Sajdera 1975, Ali, Wisby, Evans & Craig-Gray 1977], until the entire matrix vesicle and its other surfaces are encrusted with mineral. This mineral spreads through the matrix

[Anderson 1969, Anderson, Matsuzawa, Sajdera & Ali 1970, Ali, Anderson & Sajdera 1971].

It has been proposed that matrix vesicles promote calcification by concentrating the transportation of calcium and phosphorous and perhaps by removing the inhibition of calcification. The phospholipids of vesicles show a high affinity for calcium and phosphorus [Boskey 1978]. Matrix vesicles are present at sites of mineralization in the body; however, their association with mineral has almost always been observed when aqueous preparative techniques have been used. If the experimental techniques are altered to include only organic solvents, the vesicles appear to be mineral-free [Landis & Glimcher 1978].

Mineralization of osteoid involves the deposition of mineral in type I bone collagen, whereas physeal cartilage contains type II collagen. The organization of the mineral in the physis appears to be random rather than well ordered. There is, therefore, no reason to believe that the process of mineral deposition must be identical. It is logical to propose totally separate mechanisms. Gibson, Bearman & Flint in 1986 have proposed that type X collagen may be the initiator of the mineralization process.

There is a gradual change in appearance and activity of cells from the epiphyseal to the metaphyseal end of the physis. In the proliferative zone where oxygen tension is high, aerobic metabolism occurs and glycogen is stored. The mitochondria appear to form ATP and store calcium - these processes are mutually exclusive [Lehninger 1970]. In the hypertrophic zone where the oxygen tension is low, anaerobic metabolism appears to occur, and the glycogen is utilized

and disappears by about the middle of this zone. This calcium accumulation requires engergy, [Lehninger 1970] and it may be that ATP is used in the process [Shapiro & Lee 1978].

At about the midportion of the zone of hypertrophy calcium accumulation by the mitochondria ceases, perhaps because available energy is used up, [Brighton 1978] and extracellular matrix vesicles appear, with the calcium and the phosphorous eventually appearing as hydroxyapatite crystals. It is not clear whether the initial form of mineral in the matrix and matrix vesicles is hydroxyapatite or an amorphous phase, (Brighton 1978) or whether another type represents the first deposit of calcium and phosphorous. What is certain however, is that there is a regular shift to the extracellular fluid from the mitochondria of the matrix (perhaps through matrix vesicles) and that this can be followed through the various zones of physeal chondrocytes from both anatomical and temporal points of view [Howell 1971].

Cell death occurs as calcification takes place in the longitudinal septae. The horizontal septa dividing chondrocyte from chondrocyte do not calcify, and thus the mineralization is also columnated.

The organization of the chemical constituents of the physis is important. The collagen of the physis is type II [Wuthier 1969, Phillips & Young 1976, Reddi, Gay & Miller 1977]. Its chemical makeup is such that it should not calcify as readily as type I collagen of bone [Glimcher 1976]. The proteoglycans of the physis are well characterized [Rosenberg 1980, Poole 1982, Buckwalter 1983, Horton 1988]. The physis must be able to absorb energy in a fashion analogous to that of articular cartilage. The physis is part of the

load-bearing apparatus. The proteoglycan link to physeal collagen is very regular [Shepard & Mitchell 1976] and similar to that seen in articular cartilage. It is possible that some of the biomechanical properties of articular cartilage are derived from this link and from the relationship of proteoglycan to water molecules. It is logical to presume that this biomechanical advantage occurs in physeal cartilage as well.

Proteoglycans have a more important function in the physis. They are synthesized in all physeal zones, but there is a decrease in the amount and in the polymerization of these proteoglycans [Hung, Wilkins, & Blizzard 1962, Jibril 1967, Matukas & Krikos 1968, Howell, et al 1969, McConaghey 1972, Kan & Cruess, et al 1981]. There are also enzymes in physeal cartilage for degrading proteoglycans [Lucy, Dingle & Fell 1961, Dziewiatkowski 1966] that are primarily lysosomal in nature [Greenspan & Blackwood 1966, Brighton, Sugioka & Hunt 1973] and are found in the highest concentration in the zone of hypertrophic cartilage.

It has been demonstrated that proteoglycans inhibit mineralization, and it was thought that mineralization of cartilage could not proceed until they were removed or altered [Ehrlich 1985]. The observation that they disappear from the longitudinal septa as mineralization occurs has been made, [Baylink, Wergedal & Thompson 1972, Brighton, Sugioka & Hunt 1973] but immunohistochemical studies indicate an alternative aggregation rather than a true removal [Rosenberg, Poole & Pidoux 1980, Poole 1982, Horton 1988]. It has been suggested that this change in the aggregration of the proteoglycan makes the cartilage more permeable, and it is this that is responsible for the

rapid spread of mineral from the matrix vesicles, permitting calcification in the metaphyseal end of the zone of hypertrophy.

The metaphysis is thus presented with a honeycombed surface with a low oxygen tension, few living cells, and a cartilage matrix high in calcium phosphate that appears to be hydroxyapatite.

3.4.5 Metaphysis

The metaphysis begins at the last transverse septum in the epiphysis [Brighton 1978]. The loss of the transverse septae leaves the metaphysis with a tissue structured like a honeycomb. The vertical septae persist, channelling vascular invasion into the areas previously occupied by cells.

Intense cellular proliferation is seen in this area. Tritiated thymidine is taken up by endothelial cells, and by osteoprogenitor cells immediately behind the capillary buds. The capillaries invade the empty spaces and there is evidence of vascular stasis [Brighton 1974]. Occasional ruptures of capillaries are seen [Ham 1969] and electron microscopic inspection shows precipitates of material that are also consistent with stasis [Schenk, Wiener & Spiro 1968, Arsenault 1988]. Oxygen tension is low, [Brighton & Heppenstall 1971] and enzyme studies indicate that anaerobic metabolism is possibly taking place.

Osteoblasts are found immediately behind the invading and dividing capillary buds. The longitudinal septae from the physis are almost completely calcified and are called the primary spongiosium [Brighton 1978]. Rapid endochondral ossification then occurs, with the osteoblasts laying down bone on the cartilaginous bars. This is the secondary spongiosum [McLean & Urist 1961].

Resorption of metaphyseal bone must begin immediately for the metaphysis is funnel shaped and narrows to become diaphyseal bone.

3.4.6 Circumferential Structures

Two important structures surround the cartilaginous physis.

The groove of Ranvier (1873) lies at the level of the zone of reserve cells and flows into or merges with that zone. Tonna in 1961 proposed that the cells of this zone provide cells for the perichondral ring and cells and matrix for the lateral growth of the physis. The groove also contains cells that differentiate as fibroblasts, attaching the groove to the physis just proximal to the zone of reserve cells [Shapiro, Holtrop & Glimcher 1977].

The perichondral ring of Lacroix (1951), is a fibrous tube that encircles the physis at the physeal bone junction. At the proximal end it is continuous with the cells and collagen of the groove of Ranvier and this serves as an anchor point. It merges with the periosteum distally. It therefore limits the physis and provides lateral support, resisting the compressive forces across the physis [Shapiro, Holtrop & Glimcher 1977].

3.4.7 Articular Cartilage

Endochondral ossification takes place at the basal layer of articular cartilage throughout the body. This provides some growth in length, but its major function is to allow for the growth of the cartilaginous portion of joints. When the epiphyseal line is obliterated at the time of physeal closure, these basal articular layers retain some capacity to respond to stimuli and can, under pathological conditions, resume their activities.

There is an inherent polarity to articular cartilage. If a segment of articular cartilage is reversed, and its joint surface edge placed on the interior with the base of that segment facing the joint cavity, the roles of the articular chondrocytes will not reverse [McKibbin & Holdsworth 1967]. Repair potential is limited and articular cartilage repair becomes fibrocartilage under load [Campbell 1969, Bentley, Smith & Mukerjhee 1978, Trippel, Ehrlich, Lippiello & Mankin 1980, Zaleske, Ehrlich, Piliero, May & Mankin 1982].

3.5 BLOOD SUPPLY OF THE PHYSIS

The three major structural portions of the physis have their own independent blood supplies, [Dale & Harris 1958, Trueta & Morgan 1960, Brookes 1971, Chung 1976] consistent with their separate functions (Fig. 3.8).

The epiphyseal artery supplies the epiphysis (or secondary centre of ossification). Vessels entering the bony epiphysis at right angles to the surface send branches through the bony plate supporting the physis. They then branch like roots of a tree to supply the tops of the columns of cells in the proliferative zone. These vessels appear to pass through the reserve zone, and each small arteriole supplies between 4 and 10 cell columns [Trueta & Morgan 1960].

The metaphysis receives its blood supply from end arterioles that have in large part arisen from the nutrient artery. About 80% of the metaphysis is supplied by the nutrient artery [Brighton 1978]. The peripheral metaphyseal vessels supply only the outer portions.

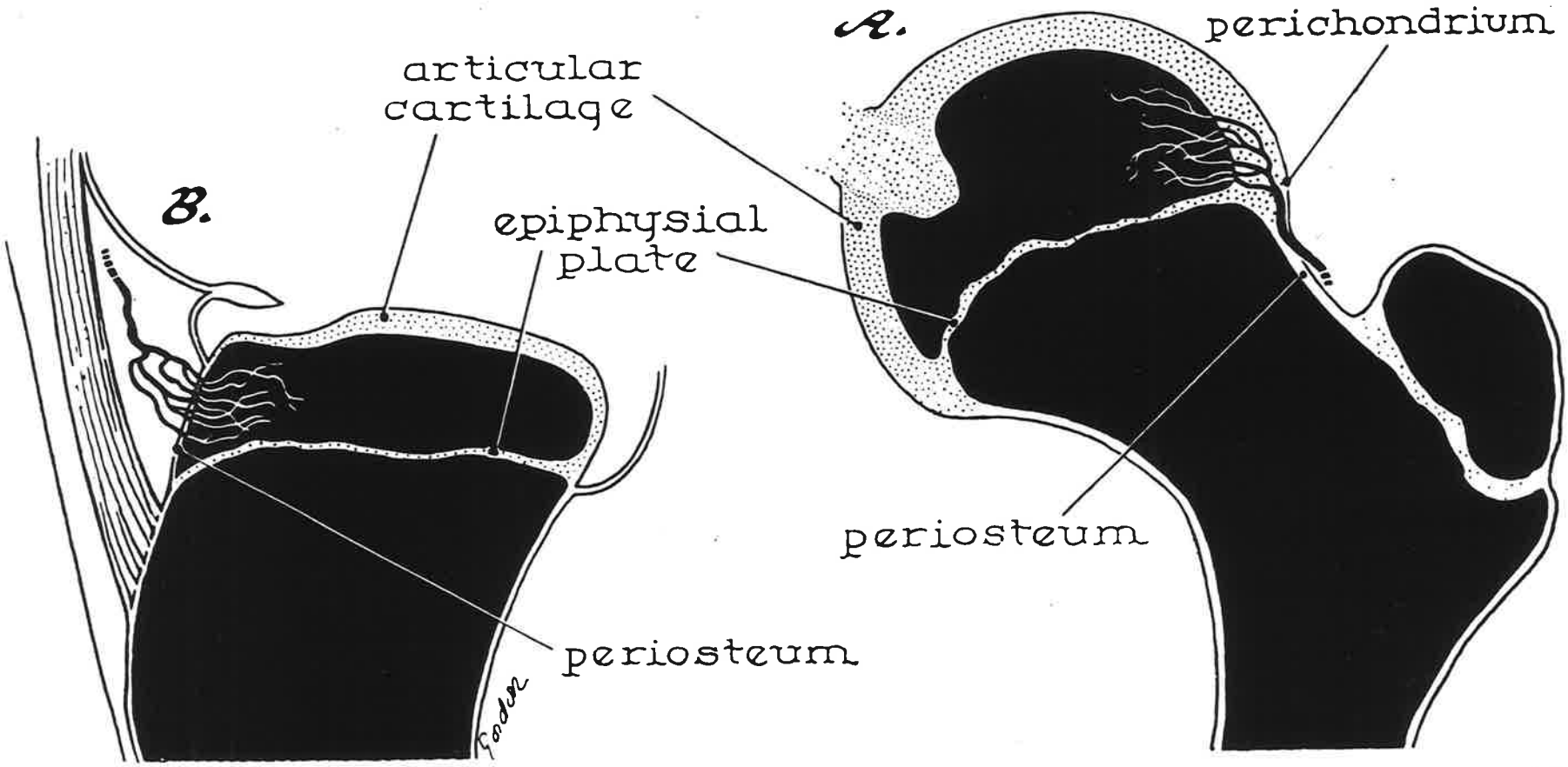
There are no anastomoses between epiphyseal and metaphyseal vessels. It is possible to interrupt one or the other experimentally, [Trueta

Figure 3.8: BLOOD SUPPLY TO THE EPIPHYSIS. There are 2 basic patterns of supply.

Type A: The epiphysis is entirely covered by articular cartilage. The blood vessels enter the epiphysis by transversing the perichondrium at the periphery of the epiphyseal plate. During epiphyseal displacement these vessels are vulnerable to rupture.

Type B: The epiphysis is partly covered by articular cartilage. The vessels enter bone by penetrating the cortex at the side of the epiphysis. In this type epiphyseal separation could occur without serious damage to vessels.

(Reproduced with permission from Dale, G. G. and Harris, W. R. Prognosis in epiphyseal separations. J. Bone Joint Surg., 40B: 117, 1958.)


A.
perichondrium
articular cartilage
B.
epiphysial plate
periosteum
periosteum

& Morgan 1960] and to observe the effects on function. Interruption of the epiphyseal vessels causes death of the physis and stops the proliferation of these cells. Interruption of the metaphyseal vessels causes failure of invasion of capillary loops from the metaphysis and a widening between the zone of mineralization and the zone of proliferation.

The groove of Ranvier and perichondral ring of Lacroix receive their nutrition from a separate set of perichondral arteries.

3.6 BIOMECHANICS:

The intrinsic strength of the physis is provided mainly by the intercellular cartilaginous matrix. In the first two zones the matrix is abundant, and the growth plate is mechanically strong. In contrast in the third zone the hypertrophic chondrocytes enlarge and there is decrease in the intercellular matrix, making this zone the weakest portion of the epiphyseal plate. This weakness is to shearing, bending and tension stresses, and not to compression [Bassett 1962, Bright, Burstein & Elmore 1974, Moen & Pelker 1984].

The fourth zone is reinforced by the addition of calcification but it is still weaker than the first and second zones. Fractures generally involve the third and fourth zones propagating a variable distance into each, not cleaving neatly between the two zones.

The weakness of the hypertrophic zone was demonstrated by Haas (1919, 1931) who found that if the periosteum of the periphery of an epiphyseal plate was incised, then the epiphysis could be detached relatively easily from the metaphysis by gentle pressure. He felt that the plane of cleavage was constant, and that it went through the

layer of hypertrophic cartilage. Amamilo, Bader and Houghton in 1985 reported on the role of periosteum in growth plate failure.

Harris (1950) and Harris & Hobson (1956) designed an apparatus to determine resistance to shear of the upper tibial epiphysis in rats, and confirmed that when the epiphysis separated from the metaphysis the plane of cleavage consistently passed through the third (hypertrophic cell) layer. The clinical significance was that the basal physeal cells remained with the epiphyseal fragment.

The region of trabecular bone formation in the metaphysis also contributes to physeal strength. The thinness and fine structure of the metaphyseal cortex in children makes it susceptible to certain kinds of injuries (e.g. compression or torus fracture) that are not seen in adults.

The cartilage of the epiphysis provides a certain shock-absorptive effect as long as it is primarily cartilaginous. In this situation the fracture producing forces are transmitted more directly into the metaphysis, resulting in torus fractures. As the epiphyseal ossification centre enlarges, this resiliency and ability to absorb stress probably are lessened, and the deformity forces are transmitted directly into the growth plate which may be sheared through the third and fourth cellular zones.

The specific three dimensional configuration of the physis in mammals is extremely variable and probably develops in response to the forces applied across each physis [Karaharju 1967, Bechtol 1973, Ogden 1979, 1980].

Because the human body is not highly specialized for rapid twisting locomotion, the configurations of the epiphyseal plates of the distal

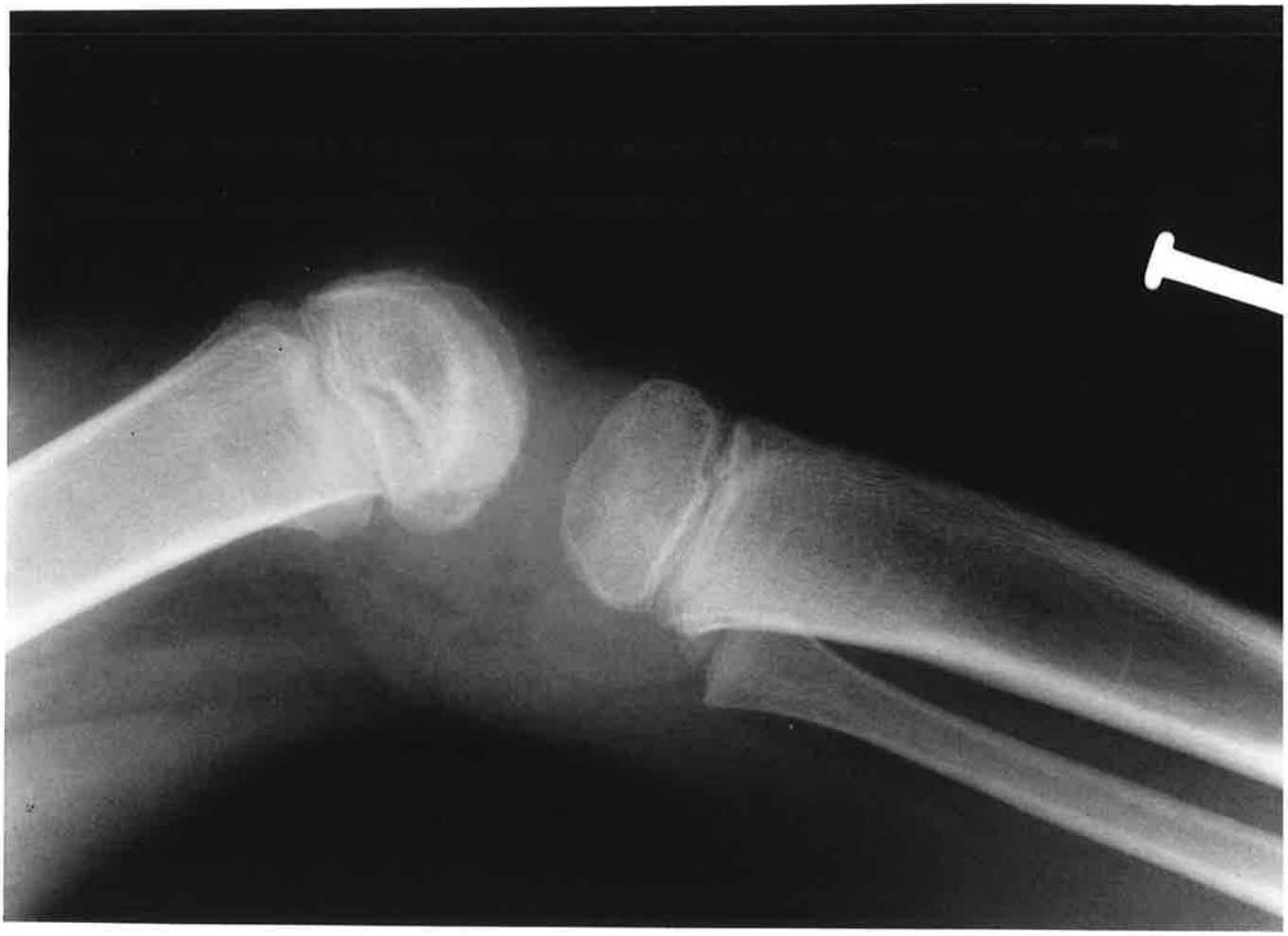
femur and proximal tibia are not as geometrically complex as they are in animals specialized for rapid running. The distal femur of the artiodactyls represents a portion of a hinge joint, and the epiphysis contains four cone-shaped projections from the metaphyseal side which fit into appropriate concave shaped areas of the epiphysis and physis. These prevent displacement in an antero-posterior/ medial-lateral direction, as well as preventing epiphyseal rotation.

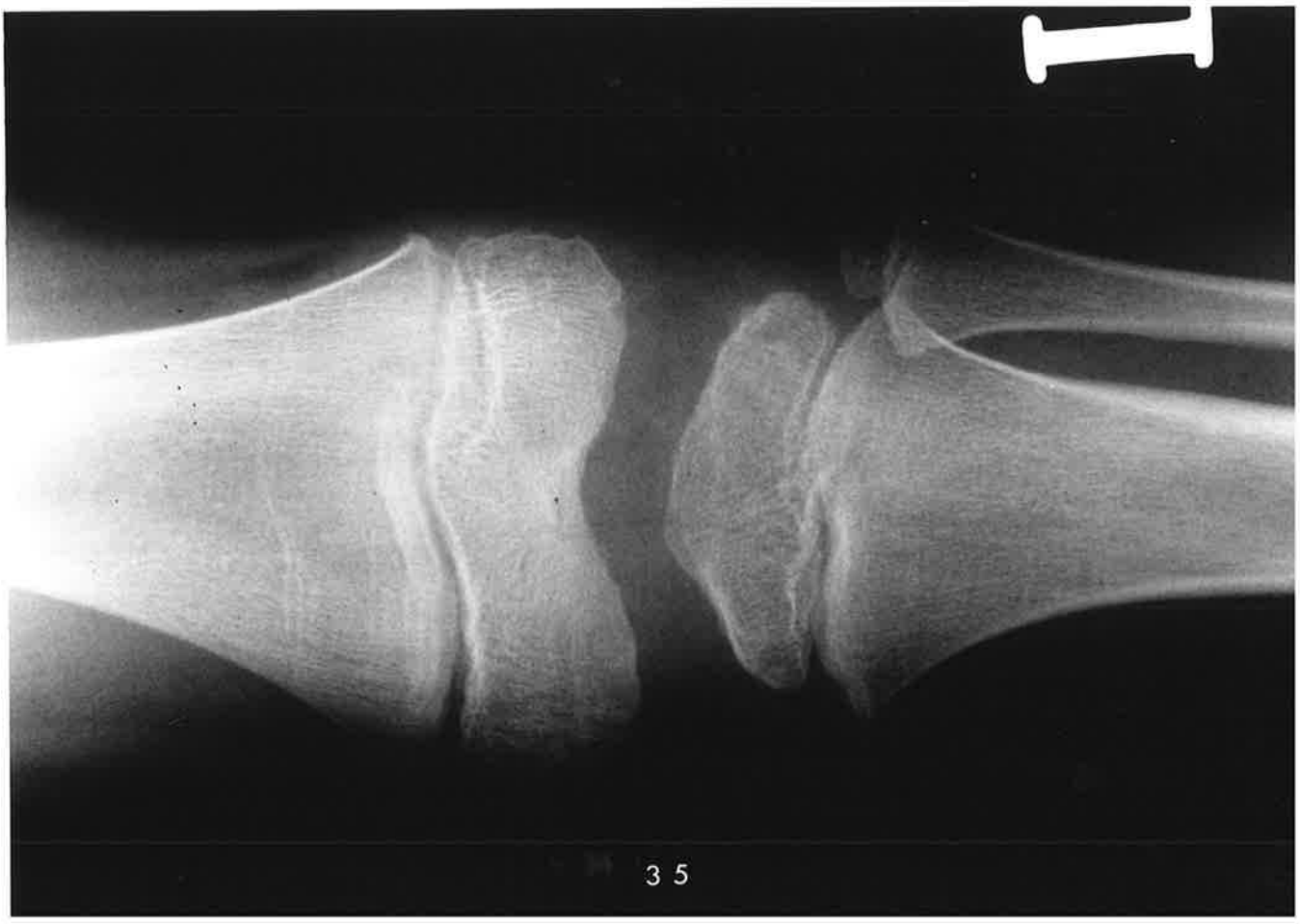
There is also a cone shaped lappet formation around the periphery of the epiphysis to further resist displacement. The importance of this is that the epiphysis is mechanically protected by these cone-shaped projections, particularly from shearing [Ogden 1982a].

However, these are secondary stabilizers to the intrinsic physeal stabilizers, and are affected by the growth plate undulations in addition to the many smaller mammillary processes. They cannot be appreciated on standard two dimensional radiographs (Fig. 3.9).

In human adolescence the cone shaped projections are more pronounced, and thus shear fracture and transverse displacement may induce fractures through multiple levels of the growth plate metaphysis and epiphyseal centre, rather than through the hypertrophic cellular zone. This irregular fracture line may pre-dispose the growth plates about the knee having a high incidence of traumatically induced physeal arrest.

Figure 3.9: **NORMAL PHYSEAL RADIOLOGY.** AP and lateral x-ray of a normal knee in a child 10 years old. The undulations of physeal contour of cone shaped projections and smaller mamillary projections of the physis cannot be easily appreciated. These structures provide secondary stability to the physis to resist shear force.





Chapter 4 DEVELOPMENT OF THE GROWTH ARREST MODEL

4.1 EXPERIMENTAL AIM:

To confirm that the technique of Phemister (1933) was appropriate to produce partial growth arrest in a sheep tibia model.

4.2 REVIEW OF THE LITERATURE:

In 1727 Stephen Hales discovered that the long bones grew in length only at their extremities. By tracing markers in the bones of animals he, and Du Hamel in 1742, and Haller in 1757, and Hunter in 1760 demonstrated a lack of an interstitial mechanism of diaphyseal bone lengthening in the normal process of bone growth.

The understanding of the mechanism of bone growth by the mid eighteenth century may be judged by the description given in the 1741 edition of Cheselden's Anatomy: "The cylindrical bones and all others whose fibres are nearly parallel, begin (to ossify) about the middle of each fibre, and thence shoot forth to their extremities; not always in continued lines, but frequently beginning new ossifications, which soon join the former; and by the continual addition to this ossifying matter, the bones increase till their hardness resists a farther extension; and their hardness always increasing while they are growing, the increase of their growth becomes slower and slower, until they cease to grow at all."

Endochondral ossification in growing bones was not described until 1836, when Miescher made reference to this basic growth process. Todd and Bowman in 1845, followed by Sharpey (1848), Leidy (1849),

and Tomes and De Morgan (1853) also studied the anatomy of the physis.

In 1858 Müller described the microanatomy of the physis, and this was further clarified by Fell (1925), Ham (1932), (1969), Dodds (1934), McLean (1940), Streeter (1949), Lacroix (1951), (1971), Maximow & Bloom (1954).

Leser (1888), Retzius (1888-89), and subsequently Retterer (1900), Dubreuil (1913), Lacroix (1951) and Sissons (1956) believed that the physis grew by the increase in size of individual cartilage cells of the zones of the plate nearest the epiphysis, where the existence of mitoses was noted.

The contrary view that interstitial growth within the bone itself was responsible for the lengthening was proposed by various authors [Hellstadius 1947, 1951]. An increase in the length of the mass of a spine fusion had been noted in immature animals and also in children [Bisgard and Musselman 1940]. In experimental studies on rabbits Odelberg-Johnson (1939) showed micro fractures of the fusion mass with subsequent fracture healing to explain this mechanism of interstitial growth.

It is now firmly established that cells of the resting zone closest to the epiphysis replicate, and as these cells mature and increase in size they pass from the maturing zones to the hypertrophic zone and become mineralized by endochondral ossification [Hunziker et. al 1987].

The mechanism of transverse, or diametric growth of the epiphyseal cartilage plate has received little attention in the literature.

Ranvier in 1873 proposed that the width of the epiphyseal plate was increased by interstitial growth. This was the same mechanism as that for longitudinal growth. In 1920 Keith, on radiological evidence from several cases of diaphyseal aclasis, supported the view of Ranvier.

In 1941 Policard and in 1947 Langenskiöld made similar conclusions after examining patients with Ollier's disease. Langenskiöld and Edgren in 1949, after inducing radiation injury to the epiphyseal plate confirmed these observations.

Solomon in 1966 postulated that the transverse diameter of the epiphyseal cartilage plate increased by appositional growth from the overlying perichondrium, and that the same source is responsible for lateral extension of the articular cartilage during growth. Rang in 1969 agreed with that conclusion and described the type 6 physeal injury.

Shapiro (1977) studied the periphery of the epiphyseal cartilage of rabbits using light and electron microscopy after labelling with ^{3}H-thymidine, ^{3}H-proline and ^{3}H-glucosamine and histochemical stains for proteoglycans and alkaline phosphatase. By these methods three groups of cells in the zone of Ranvier were identified. The second level group of cells were chondroblast precursor cells that contributed to appositional growth of the physis. The other cells were either fibroblasts merging with the periosteum and providing a periosteal tethering mechanism, or osteoblastic progenitor cells deep

in the groove providing a cuff of bone surrounding the epiphyseal growth plate and the adjacent metaphysis.

In 1867 Ollier performed a series of experiments to observe the effects of injury on the epiphyseal plate. Different degrees of injury were created. He found that superficial linear incisions across the epiphyseal plate of skeletally immature rabbits and cats had little influence on bone growth, but that deep incisions led to growth arrest. Multiple needle punctures of the plate did not affect growth. Ollier also showed that experimental separation of the epiphysis did not influence growth if the epiphysis was immediately replaced.

In 1873 Bidder proved that a partial lesion of the epiphyseal plate was followed by the formation of a bone bridge between the metaphysis and epiphysis. The bone bridge caused retardation and degenerative changes in the physis starting from the side of the injured area. His observations were confirmed by Nové-Josserand (1894).

In 1878 Vogt did not find any retardation of growth in goats and sheep if epiphyseal separation occurred at the natural line of cleavage of the epiphyseal and metaphyseal junction. However, if the epiphysis was deeply lacerated at the time of separation, then growth retardation could occur.

Using rabbits Jahn noted in 1892 that if transversely oriented discs of the epiphyseal plate 0.5 to 1.0 millimetres in diameter were removed from the plate at the epiphyseal end of the physis columns, then these columns were unable to reproduce themselves.

Haas in 1917 and 1919 analysed the growth disturbance in the metacarpal and metatarsal bones of dogs produced by surgical incisions, crushing, and other injuries of the centre of the epiphyseal plate.

Gatewood and Mullen (1927) and Banks (1941) showed that if the damage was confined to the epiphysis and did not involve the epiphyseal plate, then there was no effect on growth.

Friedenberg in 1957 found that, in attempts to prevent bone deformity, he could not control the reactive osteogenesis secondary to the trauma. Resection of sections of epiphysis in rabbits resulted in premature arrest, probably as a result of the resection of the regenerative zone of Ranvier and ossification of the ring of Lacroix. He noted that, in animals with minor peripheral deformities, micro-fractures of the osseous bridges were constantly induced. Johnson and Southwick in 1960 confirmed this micro-fracture effect as a form of metaphyseal remodelling.

Ford and Key in 1956 perforated the epiphyseal cartilage of young rabbits with a 1/8" drill. This did not cause major shortening in non-osseous or fibrous bridges. When larger drill sizes were used shortening became more marked as a result of osseous bridges.

Harris and Hobson (1956) studied the displaced proximal femoral epiphysis in rabbits, finding a line of cleavage in the zone of hypertrophic cartilage cells. In monkeys, Dale and Harris (1958) confirmed that separation occurred through the junction of hypertrophic cartilage cells and the calcified portion of the growth

plate. This substantiated the work of Haas (1931), in which he attempted epiphyseal cartilage plate transfer.

Campbell, Grisolia and Zanconato (1959) studied the histological effects and growth disturbance caused by surgical trauma to the epiphyseal plate in immature dogs.

The summary of the findings is represented in Fig. 4.1.

It was shown that small diameter central defects to the physis up to 5 x 0.045 centimetre drill sizes either with or without pins caused minimal growth retardation. If a threaded pin or larger diameter hole of 5/32" was used then physeal arrest occurred. With peripheral defects, if the metaphysis and epiphysis of the displaced bone segments became avascular or denatured by alcohol, there was maximal growth retardation.

The effect on longitudinal growth of the bone by metal pins and wires transversing the epiphyseal plate has been investigated by several authors [Haas 1945, 1950, Gelbke 1951, Siffert 1956, Key and Ford 1958, Campbell, Grisolia and Zanconato 1959, Ford & Canales 1960, Kolesnikov 1967]. It has been shown that smooth pins cause least disturbance.

Many authors have explained the vulnerability of the physis to other than mechanical trauma. Temporary ischaemia caused by moderate deviation of the epiphyseal plate may lead to avascular necrosis, thereby causing bone bridge formation between the epiphysis and the metaphysis [Trueta & Amato 1960, Troupp 1961, Brashear 1963, Young 1966]. It has also been possible to create physeal injuries by using

Figure 4.1: **SUMMARY OF GROWTH DISTURBANCE CAUSED BY SURGICAL TRAUMA TO THE EPIPHYSEAL PLATE IN IMMATURE DOGS.**

For central growth arrest the critical (minimal) injury was a 5/32" drill hole, whereas for peripheral retardation the requirement was avascularity of the displaced bone segments.

(Reproduced with permission from Campbell, J. C., Grisolia, A. and Zanconato, G. The effects produced in the cartilaginous epiphyseal plate of immature dogs by experimental surgical traumata. J. Bone Joint Surg., 41A: 1221, 1959.)

Group	*Operation*	*Line Drawing of Operation*	*No. of Operations*	*General Effect on Growth*
1	Resection of the margin of the epiphyseal plate, epiphysis, and adjoining metaphysis		6	Minimum angulation
2	Longitudinal osteotomy through the distal radial and femoral epiphysis crossing the plate in a plane perpendicular to it			
	a. No internal fixation		5	Minimum retardation
	b. Internal fixation—one screw transversely across the metaphysis		15	Minimum retardation
	c. Detachment of all soft tissues from fragment, with replacement and fixation		5	Maximum retardation
	d. Detachment of soft tissues, insertion in 90 per cent alcohol for ten minutes, replacement, and fixation		3	Maximum retardation
3	Longitudinal osteotomy in the metaphysis of the radius extending distally to the epiphyseal plate with separation across the plate in the line of cleavage		15	Variable retardation (none to moderate)
4	Drilling of holes longitudinally across the distal epiphyseal plate of the radius and femur			
	a. One hole, one-quarter of an inch in diameter		2	Maximum retardation
	b. One hole, five-thirty-seconds of an inch in diameter		13	Minimum retardation
	c. One hole, five-thirty-seconds of an inch in diameter, with the insertion of beeswax		5	Minimum retardation
	d. Eight to ten holes, 0.45 millimeter in diameter		5	No retardation
5	Longitudinal insertion of cortical-bone graft (homogenous), five-thirty-seconds of an inch in thickness, through a drill hole across the distal epiphyseal plate of the femur		8	Arrest
6	Longitudinal insertion of smooth metallic pins extending from the articular surface of the epiphysis into the metaphysis of the distal part of the femur and radius			
	a. Steinmann pin, five-thirty-seconds of an inch		14	Minimum retardation
	b. Five Kirschner pins, 0.045 centimeter		4	Variable retardation (none to minimum)
7	Longitudinal insertion of threaded metallic pins (one-quarter of an inch and five-thirty-seconds of an inch) from the articular surface of the epiphysis into the metaphysis of the distal part of the femur		17	Arrest

x-ray irradiation [Perthes 1903, Engel 1938, Reidy et al 1947]. Bone-seeking radio-isotopes result in similar changes [Ray & Thompson, et al, 1956]. A diet rich in Strontium^{90} is able to disturb ossification and cause degenerative changes in the physis [Storey 1965]. External physical changes such as frost bite probably cause damage as a result of vascular occlusion [Lindholan, Nilsson & Svartholm 1968].

Growth arrest produced by a bone bridge between the epiphysis and metaphysis is used in clinical practice after the method of Phemister (1933).

Irrespective of the aetiological factor causing a bone bridge, it is evident that it constitutes a mechanical impediment to growth, and the size and density of the bone bridge are in direct proportion to the growth disturbance [Ollier 1867, Bidder 1873, Nové-Josserand 1894, Nakahara 1909, Haas 1919, Phemister 1933, Ford and Key 1956, Friedenberg and Brashear 1956, Friedenberg 1957, Campbell, Grisolia and Zanconato 1959, Johnson and Southwick 1960, Heikel 1960, 1961, Barash and Siffert 1966, Österman 1972].

The percentage of physeal area that has to be damaged to create a fusion has not been documented. For this reason, for the purpose of this work, it was decided to use an animal model of peripheral growth plate damage by creating bone bridges after the clinical method of Phemister. A piece of peripheral bone, including epiphysis, physis, and metaphysis is reversed. It was recognised that small bone bridges have caused only minimal growth disturbances.

4.3 MATERIALS AND METHODS:

4.3.1 Animals:

This series of experiments was undertaken on Merino/Suffolk cross-bred sheep since similar experiments on limb lengthening have been performed using sheep as a model [Monticelli 1981*a*, 1981*b*, DePablos 1986]. Hecker in 1983 indicated that possibly only for the dog does there exist a wider range of experimental surgical techniques.

An animal of sufficiently large skeleton is important when considering the need for reversal procedures of deformity. In 1978 Sledge and Noble reported that, experiments failed in a rabbit model with a distraction frame to reverse deformity, due to fractures of the long bones through the pin sites on the bone, and so recommended studies on larger animals.

Bisgard and Martenson (1937) showed that in goats a Phemister technique (1933) of inducing a bony deformity could be satisfactory, but had some difficulty in inducing a complete growth arrest if the remnant plate was left undisturbed at the time of reversal of the epiphyseal/physeal/metaphyseal bone segment.

Bright (1972, 1974, 1981) used puppies for a series of experiments involving reversal of bone arrests. During the course of the author's experiments, it was considered that the use of puppies would not permit completion of the experiments because of the anti-vivisection issue.

Other larger animals such as dogs and pigs have a longer age to maturity (up to 6 years), but sheep rapidly mature to adult height by 9-10 months even though full skeletal maturity does not occur until 2 $^1/_2$ years [Newton-Turner 1953, Allden 1965]. Sheep have a potential lifespan of 15-20 years in the laboratory. On farms 5-8 years is suitable for long term experimentation after surgical modification. The relatively rapid maturation would allow study of the long-term effects of any physeal manipulation on premature physeal closure.

The pig is an alternative large laboratory animal. Two books on this subject have been published "The Pig as a Laboratory Animal", [Mount and Ingram 1971] and "Swine in Biomedical Research", [Bustad and McClellan 1966]. Pigs have many similarities to man, particularly in their digestive system and skin, but they can grow to an unmanageable size and can be most unpleasant and uncooperative animals in the laboratory. These attributes restrict their use.

Histologically, the characteristics of sheep bone have been found to be similar to man [Perren 1969, Monticelli 1981a, 1981b, Peltonen 1984, DePablos 1986]. Experiments on larger animals having longer periods of immaturity are more applicable than experiments on rabbits or mice. This is because there are risks of failure by producing a fracture in smaller bones and also the growth potential of the physis can continue throughout adult life in some species such as the rat.

In 1981 Seinsheimer and Sledge showed that physeal activity was age and site dependent. Therefore, to obtain lambs of known neonatal age, a program of animal husbandry was established [Foster et al 1984]. This ensured consistency between experimental animals for comparative rate of physeal activity.

For the provocative initial operative procedure of physeal damage at six weeks of age the lambs were transported with the ewe and weaned in the post-operative period.

The hind limb was selected as it has no fibula. It represented the tibia as a single bone [Sisson and Grossman 1953]. The tibia has a single physis proximally to contribute to angulatory and longitudinal growth potential. The forelimb bones were not used because the lambs would be hindered in mobility, particularly in standing from a lying position where kneeling is often required.

4.3.2 Operative Technique:

6 Week old lambs had a general anaesthetic induced by Pentothal and Fluothane/Nitrous Oxide technique.

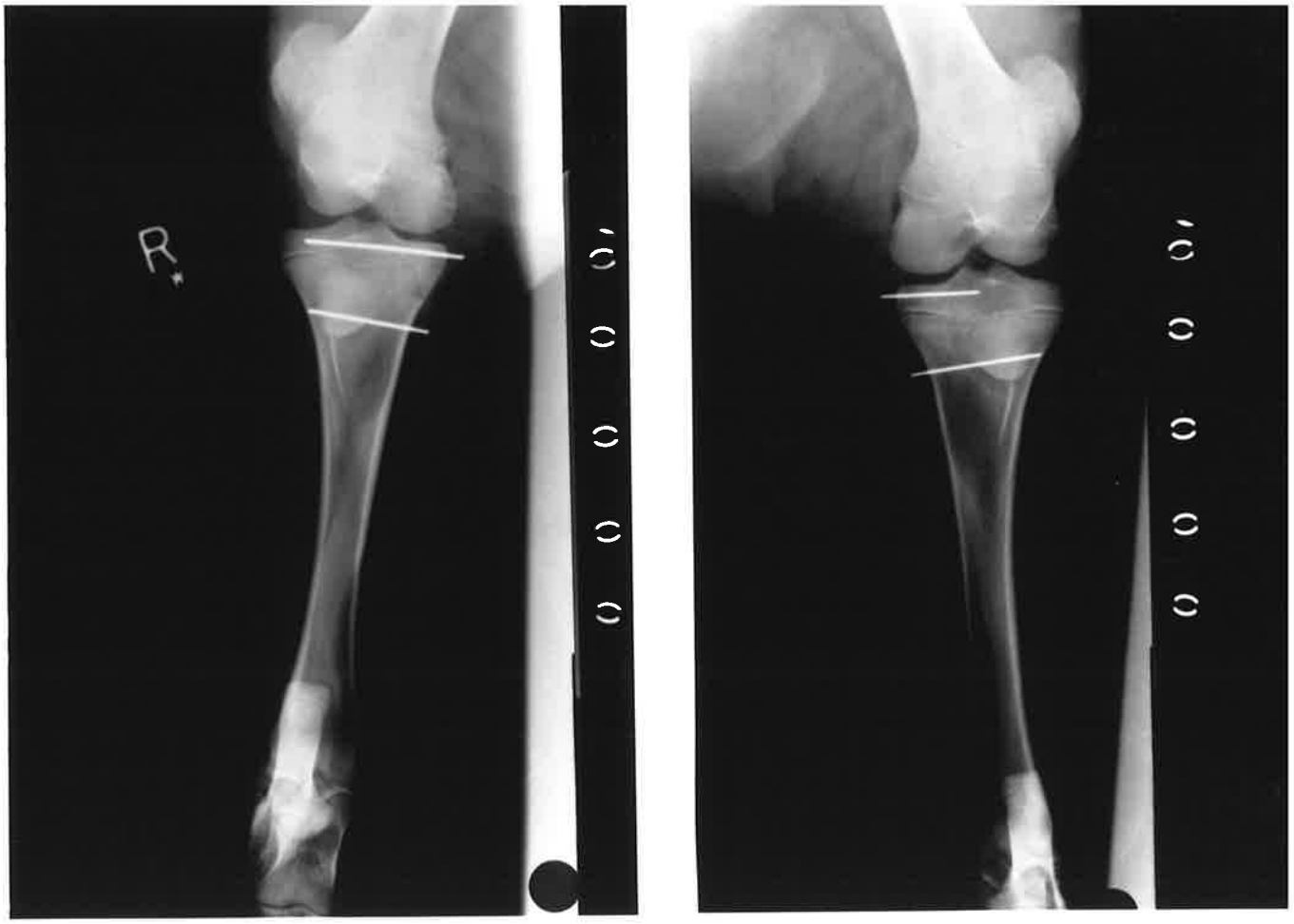
The experimental hind limb was randomly chosen. The contralateral limb had a sham operation as a control. A sterile free drape of the limbs was done. A medial longitudinal incision exposed the subcutaneous tissue, and then an arthrotomy of the knee joint was performed to determine the level of the proximal physis of the tibia. The physis was inspected directly using binocular loupe magnification of x 2.5.

A 0.062 mm. Kirschner wire (K-wire) was placed in the epiphysis and a K-wire marker was also placed in the diaphysis a known distance from the proximal pin. In earlier experiments this distance varied from animal to animal. After the initial pilot group of 5 animals an F-shaped template of confirmed 20 mm. separation also ensured parallel placement of marker pins (Fig. 4.2).

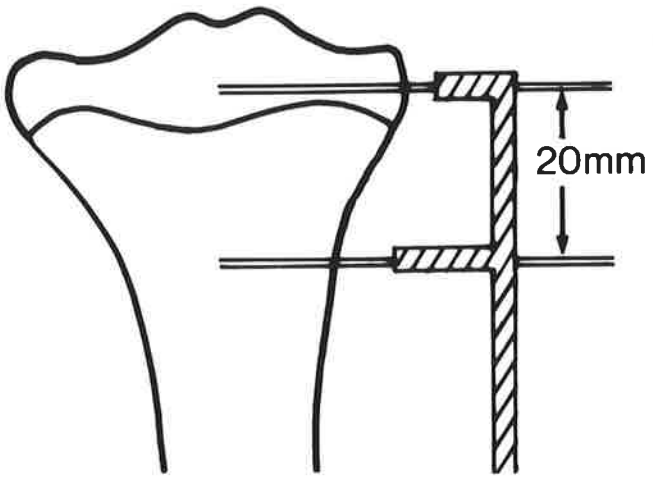
Figure 4.2: K-WIRE MARKER PLACEMENT.

Figure 4.2.1: A.P. x-ray shows K-wire marker pins in situ on left and right tibia post operatively. The K-wires remain in situ and the pin angulation gave a measure of bone varus angulation.

Figure 4.2.2: The F shaped template helped to ensure parallel placement of marker pins at a known 20 mm gap. It confirmed that the rate of proximal tibial physeal growth was 1mm/week. (unpublished data)



METHOD OF PIN PLACEMENT



"F" template

These radio-opaque markers remained in place throughout the experiment to allow measurement of length and angulation. Intra-operative use of the Image Intensifier (Toshiba 6XT) confirmed the pin positions in each case.

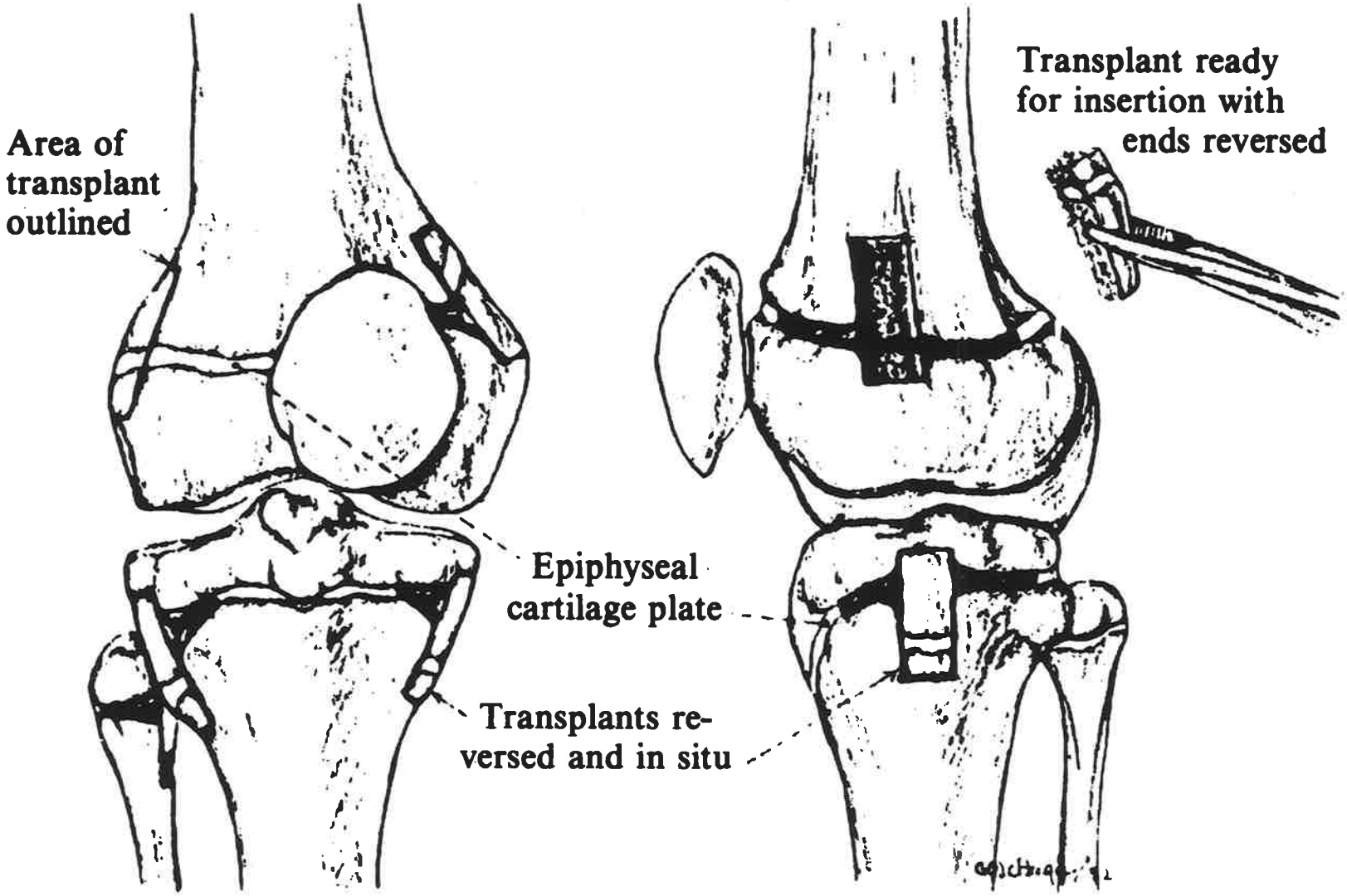
Using the technique of Phemister, a single partial peripheral growth arrest was undertaken in each experimental tibia. A 1 sq. cm. defect of the growth plate was excised *en bloc* using a 1 sq. cm. box chisel and transposed (Fig. 4.3). To quantitate the effects of larger bone bridges, 2 sq. cm. and 3 sq. cm. defects were created by the same method.

In all experiments no further physis was exposed or removed other than the single operative field. The soft tissues were sutured over the bone, and the skin closed with a continuous 0 dexon suture (3.5 metric green braided polyglycolic acid sutures coated with polaxamer 188 Davis & Geek) after Betadine (Povidone - iodine solution B.P. Delta-West Ltd., Western Australia) solution was placed in the wound to decrease the risk of infection. Also to limit the risk of wound infection a 2 ml. b.d. intramuscular 3 day peri-operative course of Streptopen antibiotics was given. (Procaine Penicillin 200 mgs/ml and Dihydrostreptomycin 200 mgs/ml, Glaxo.)

On the contralateral limb a control sham operation was performed. This involved an incision of the skin and a knee arthrotomy to expose the proximal tibia. A longitudinal single incision of the periosteum between the epiphysis and the metaphysis was done and K-wire pins were placed into the metaphysis and epiphysis as previously described.

Figure 4.3: **EPIPHYSEAL ARREST BY PHYSIODESIS, ACCORDING TO PHEMISTER.** A 1 sq. cm. fragment of growth plate was excised en bloc using a box chisel and transplanted with ends reversed.

(Reproduced with permission from Phemister, D. B. Operative arrestment of longitudinal growth in bones in the treatment of deformities. J. Bone Joint Surg., 15: 1, 1933.)


Area of transplant outlined
Transplant ready for insertion with ends reversed
Epiphyseal cartilage plate
Transplants reversed and in situ

At the finish of the operative procedure under anaesthetic the male lambs were castrated, to allow ease of handling post-operatively and all animals were crutched to diminish risk of stock loss.

On recovering from the anaesthetic, the lambs walked fully weight bearing and were transferred to the farm for on-going observation after a 3-5 days post-operative care in the animal house.

At three months post-operation, animals were killed by nembutal overdose and fusion confirmed by histological examination.

4.3.3 Radiological Techniques:

To avoid any variation in magnification all x-rays were taken in the same standardized manner. The hind limbs were held in neutral rotation for the antero-posterial view, and full external rotation for the lateral view. The distance between the tube and the x-ray plate was standardized by using a perspex x-ray cassette holder, upon which the lamb limbs were held, and the focal distance of the tube was 80 cm. Post-operative x-rays confirmed the marker positions.

Initially for the pilot animals the x-rays were repeated in all lambs at 2 weekly intervals. The sheep were given a Pentothal anaesthetic for these x-rays. This frequency of radiological confirmation of fusion proved unnecessary and x-rays were then taken at 6 weekly intervals to monitor the progression of peripheral physeal fusion.

The state of the epiphyseal plate, the longitudinal growth of the bone, changes in varus deformity, and the site of the provocation procedure were recorded on the x-rays.

4.3.2 Bone Deformity Index:

(i) Bone Lengthening Ratio:

In all x-rays the pin distance and pin angle was measured, as was the bone length and bone angle (Fig. 4.4). Serial measurements of bone length, pin-separation distance, bone angle and pin angle were undertaken (see Appendix I).

Despite using a standard radiographic technique there were variable magnification effects from each measurement taken at different times. The magnification effects remained consistent for the same day when both limbs were radiographed in the same way. Thus, to eliminate the magnification errors, a ratio of bone length and pin distance was calculated as suggested by Dr. N. Fazzalari (Fig. 4.5).

The bone lengthening ratio is equal to $\frac{\text{Pin Distance}}{\text{Bone Length}}$

Therefore each bone may be calculated as;

$$\frac{\text{Pin Distance}}{\text{Bone Length}} = \frac{C_1D_1 \times \text{magnification factor 1}}{A_1B_1 \quad \text{magnification factor 1}}$$

Eliminates magnification 1

i.e. $\frac{C_1D_1}{A_1B_1}$ = BONE LENGTHENING RATIO TIBIA 1

AND $\frac{C_2D_2 \times \text{magnification factor 2}}{A_2B_2 \quad \text{magnification factor 2}}$

Eliminates magnification 2

i.e. $\frac{C_2D_2}{A_2B_2}$ = BONE LENGTHENING RATIO TIBIA 2

The ratio of: $\frac{\text{BONE LENGTHENING RATIO OPERATED LEG}}{\text{BONE LENGTHENING RATIO CONTROL LEG}}$

Figure 4.4: RADIOLOGICAL BONE DEFORMITY.

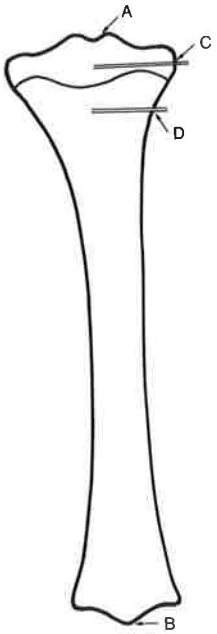
Figure 4.4.1: Measurements of bone length (A-B) and pin distance (C-D).

Figure 4.4.2: Measurement of Bone Angle: The bone angle is defined as the shaft/articular surface angle.

Figure 4.4.3: Measurement of Pin Angle: Either directly or using the Cobb method (1948).

Figure 4.5: MEASUREMENT OF BONE LENGTHENING RATIO: To eliminate magnification a quotient of the ratio of $\frac{\text{pin distance}}{\text{bone length}}$ of the operated leg was divided by the ratio of $\frac{\text{pin distance}}{\text{bone length}}$ of the control leg. Initially, this ratio was 1.000 and fell to 0.500 as arrestment occurred.

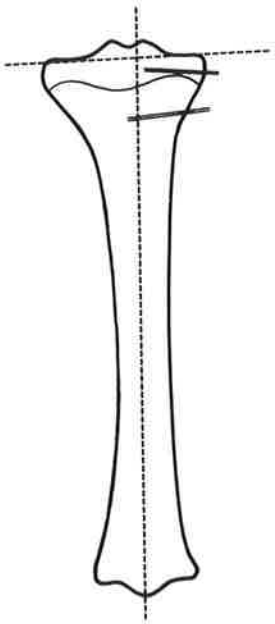
MEASUREMENT
OF BONE LENGTH
AND
PIN DISTANCE



A - B = Bone length
C - D = Pin distance
C - D = 20mm with template measurement

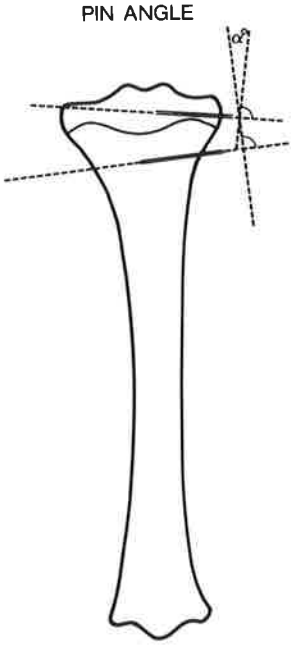
MEASUREMENTS OF

BONE ANGLE



On the X-rays lines were projected along the shaft of the bone and across the tibial plateau, in order to measure the bone angle.

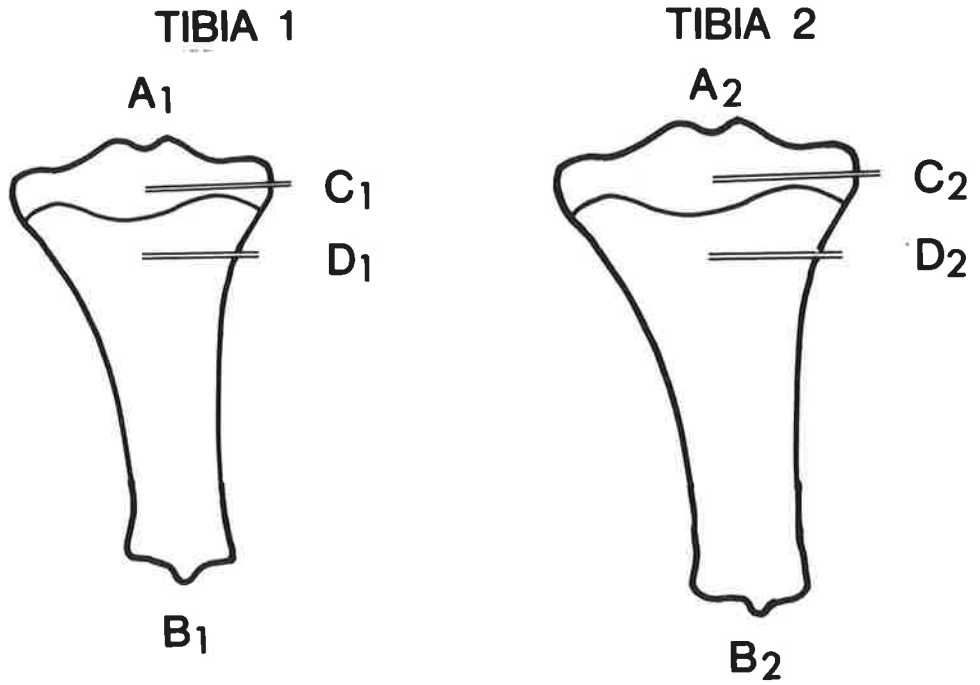
PIN ANGLE



Lines were projected through the pins in order to measure the pin angle [α°].
(After the method of Cobb)

BONE LENGTHENING RATIO

Elimination of Magnification Effect



defined the parameter of final bone length. Initially this is 1.000 or slightly less than or greater than 1.000 as determined by biological variance.

(ii) Bone and Pin Angulation:

The bone angle and pin angle measurements also vary with different degrees of rotation [Brodin 1955, Österman 1972]. To minimize this error in measuring the deformed bone axis for the bone angle measurement the pin angle was taken as the most accurate angle of bone deformity. The radio-opaque pins that were initially parallel could be measured, with protractors directly, or using the method of Cobb (1948) to measure the deviation (Fig. 4.4).

This gave a radiological deformity index of two parameters.

1. Bone lengthening ratio.
2. Pin angulation.

The angulation and ratio determined the radiological evidence of a fusion across the epiphyseal plate.

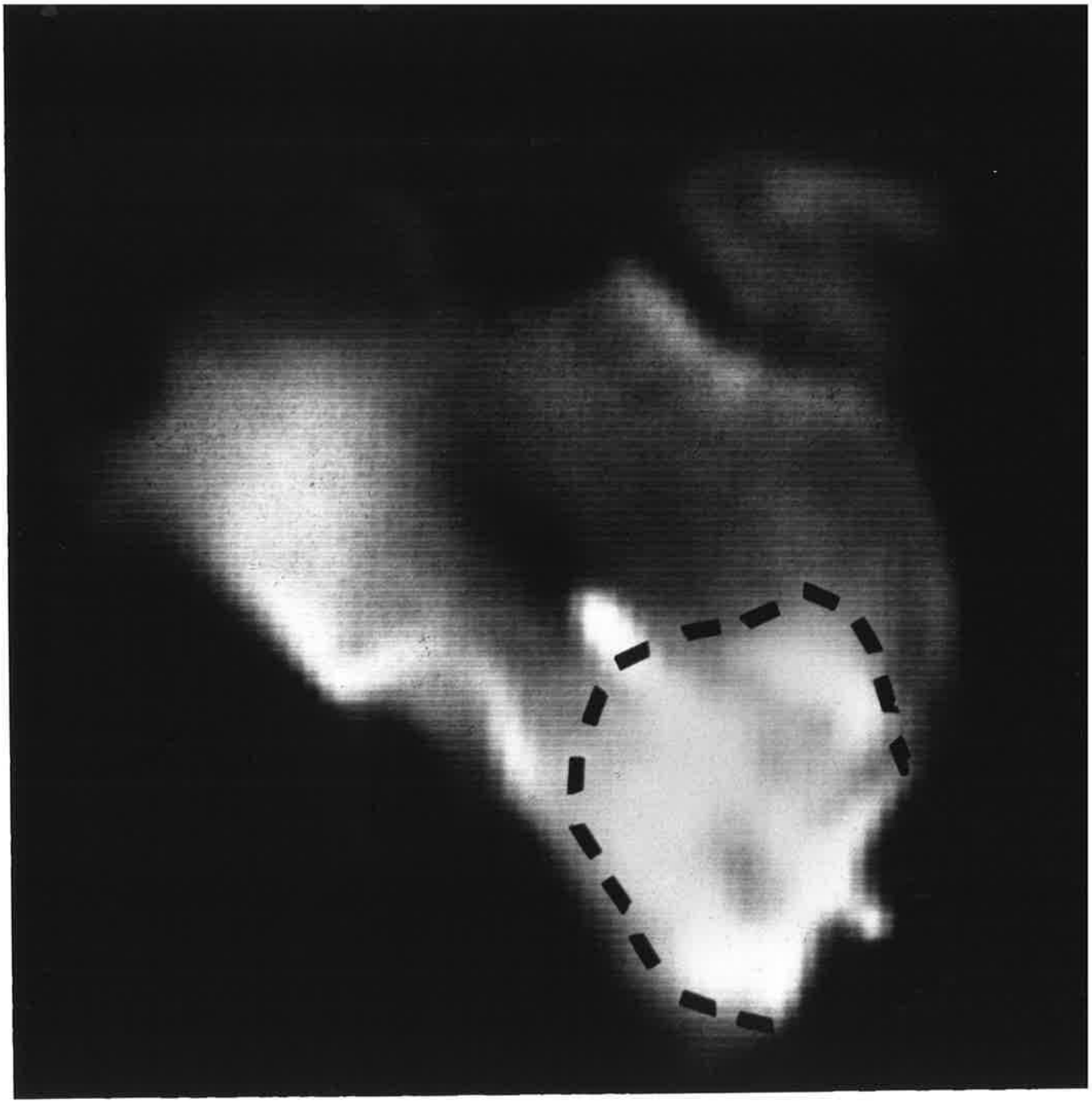
(iii) C.T. for measurement of area of fusion mass:

Once cleared of other tissue the bones were sent for computerized tomographic section (G.E. 9800) at Flinders Medical Centre. 3 or 4 x 1.5 mm. thick transaxial abutting cuts at 5 mm. spaced intervals were performed commencing at the superior marker pin in the epiphysis. Results were recorded on a floppy disc (F.D. 34-9000-18613 * Datalife by Verbatim) and also hard copy x-ray of the transaxial scan of the bone at the level of the epiphyseal plate (Fig. 4.6). The less dense physeal cartilage could be seen in relation to the cortical and cancellous bone. At the level of the operation the tissue reaction

Figure 4.6: **COMPUTERIZED TOMOGRAPHIC SECTION OF THE TIBIAL PHYSIS.**

The area of fusion mass is calculated as a percentage of the total area immediately adjacent to the proximal epiphysis at the level of the physis. The density of the bone fusion is greater than 75 Hounsfield units and is outlined by the broken line in this figure.

By computerized planimetry on sections of the tibia the area of physeal disruption was calculated as a percentage of the whole physeal area.



could be separately identified. The density of the tissues was recorded in Hounsfield Units (H.U.) by the scanner. The density of fibrous tissue ranged from 20-75 H.U. and bone 75 H.U.

The area of a transverse section of bone at the physeal level was estimated using a planimeter. The average of three separate measurements was taken of the adjudged area of bone bridge damage at the physeal level. This gave an area of the defect as a percentage of the physeal area as a quantitative index of the extent of the peripheral growth plate damage necessary to produce a partial growth arrest.

4.3.5 Examination of Specimens at Autopsy:

All specimens were taken at autopsy, and soft tissues from the lower limbs removed below the knees. Right & left limbs were separately marked and immediately placed in 10% neutral buffered formalin. Measurements of bone length and pin distance were taken with calipers (Fig. 4.7).

4.3.6 Histological Quantification:

All bones had whole coronal sections of the proximal tibia of the operated and non-operated side prepared (Fig. 4.8).

The marker pins were used to identify the coronal level of provocation of the fusion and also the level of the sham control side.

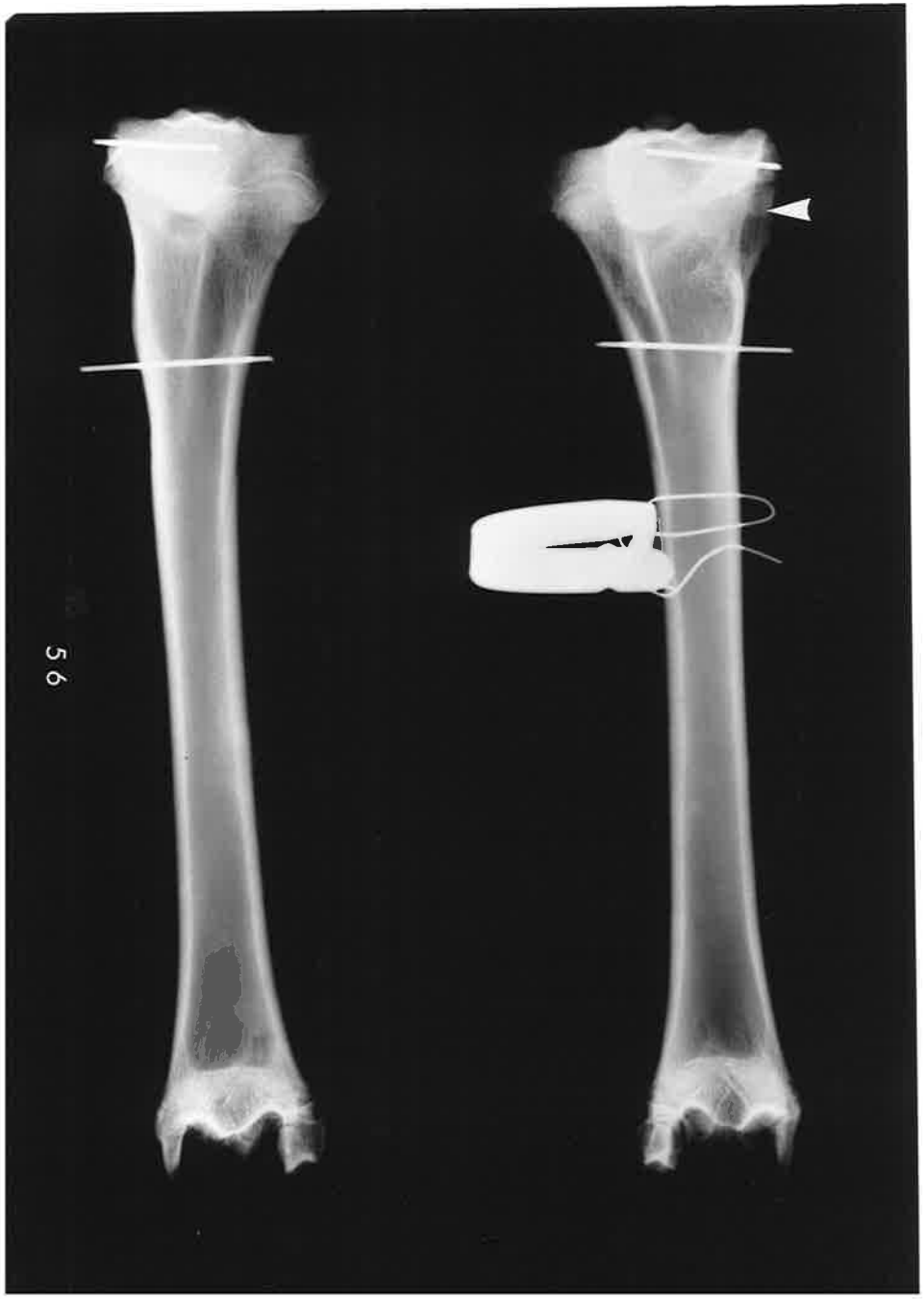
After fixation in 10% neutral buffered formalin a band saw was used to cut coronal sections of the bone at the marker pins. Occasionally because of circumferential bone growth, it was difficult to find the marker pins. To eliminate the potential for sectional error the

Figure 4.7.1:

MEASUREMENT OF PIN DISTANCE AT AUTOPSY.

Calipers measured the distances after removal of the soft tissues and excessive bone growth over the pins.

Figure 4.7.2: Specimen fine grain x-ray at autopsy. This shows evidence of partial fusion. ▶



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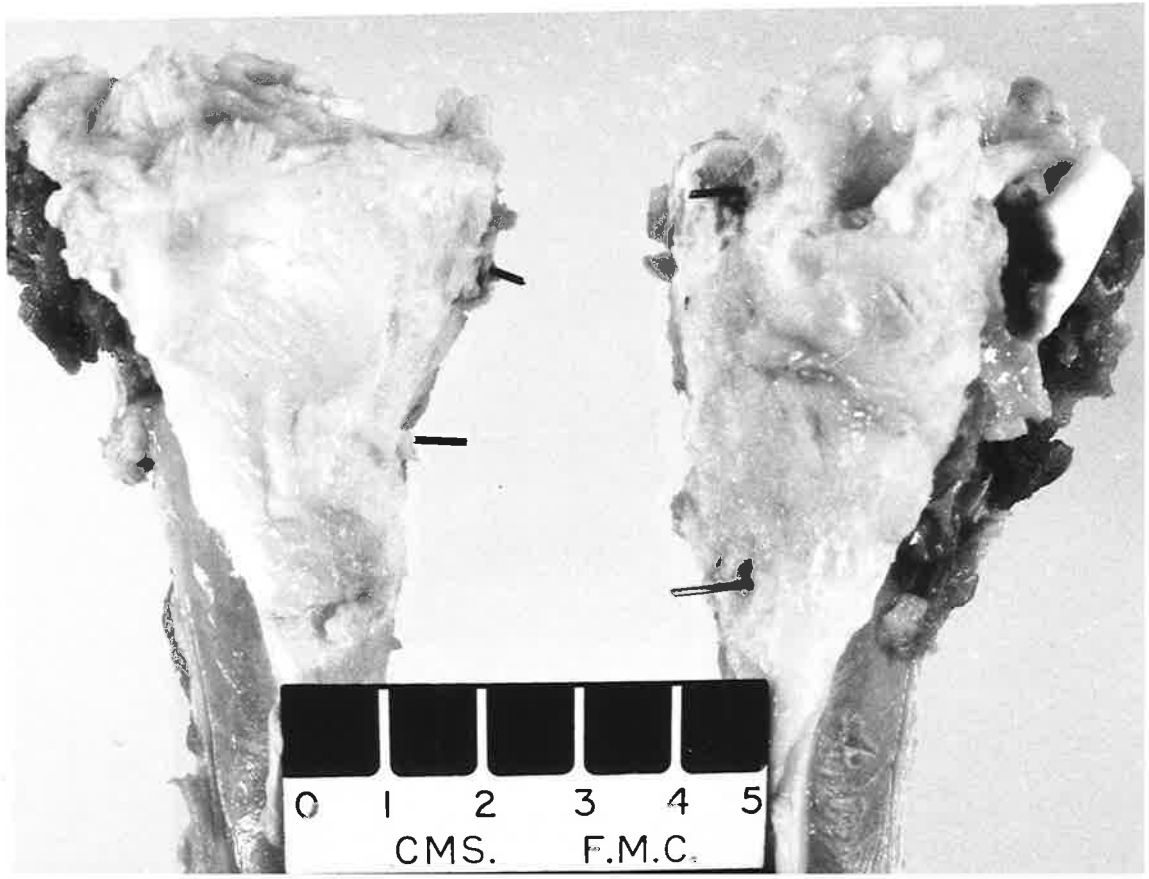
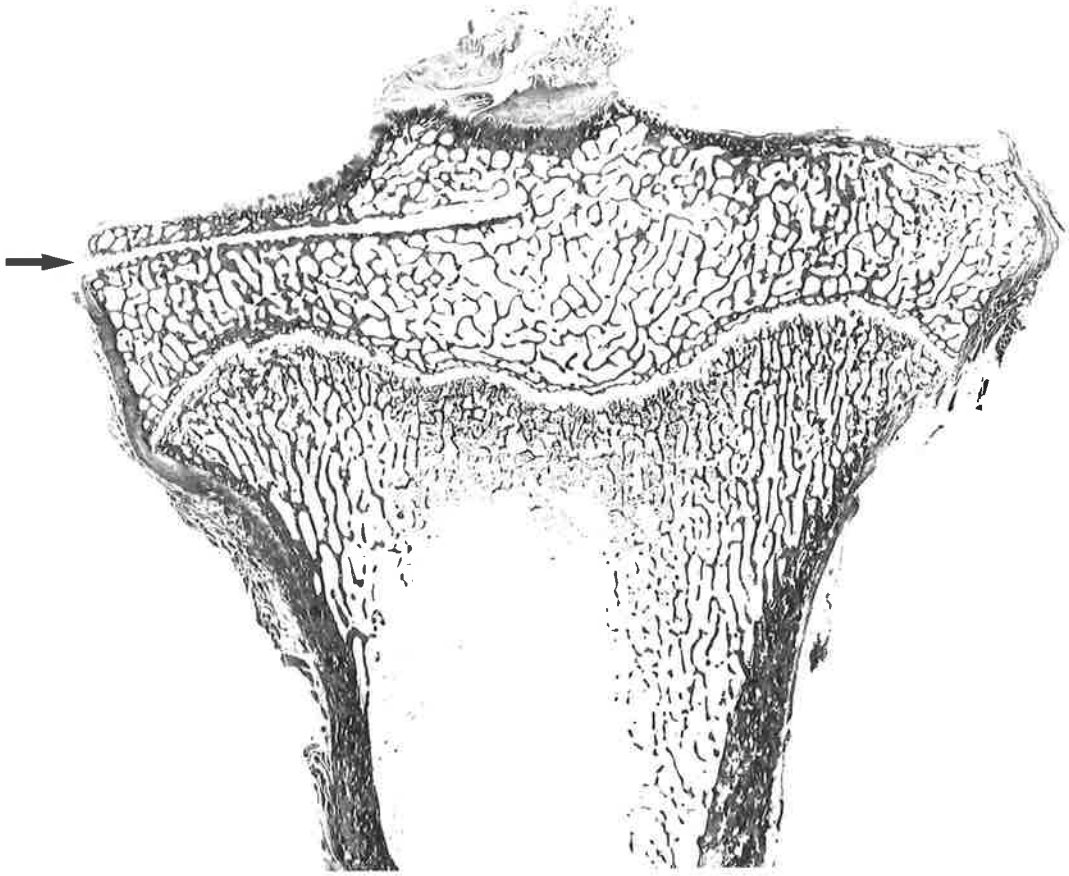


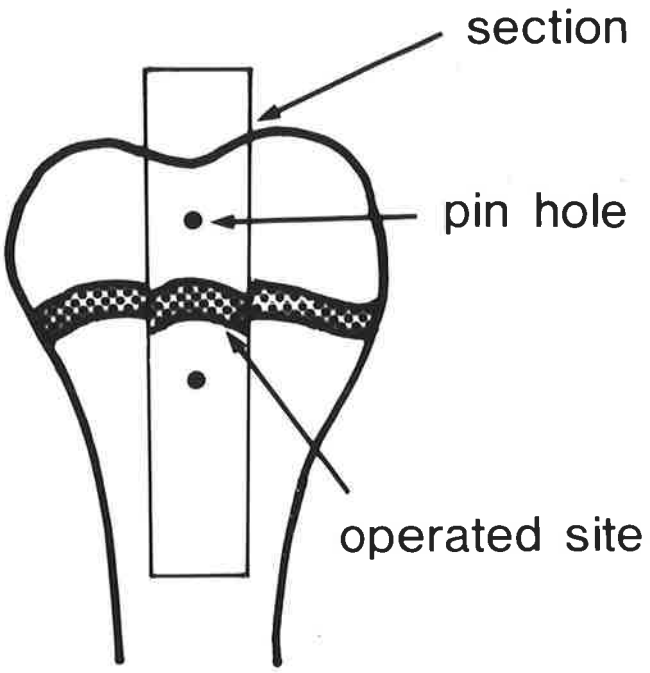
Figure 4.8: PREPARATION OF HISTOLOGICAL SECTION.

Figure 4.8.1: Normal proximal tibia (control side) of Animal 7. The pin tracts in the epiphysis → (or diaphysis) ensure that the section has been taken through the appropriate level. (x 3)

Figure 4.8.2: Coronal section of the proximal tibia with a band saw cut 5 mm wide at the level of the marker pins.



HISTOLOGICAL SECTION



coronal sections were placed in decalcification solution and then the K-wires removed before cutting sections to prevent microtome damage.

Decalcification was carried out using 10% EDTA (ethylene, diamine tetra-acetic acid) in 7% Hydrochloric Acid. Fine grain radiographs checked the degree of decalcification.

Sections were embedded in paraffin wax. Three x 5*u* thick sections at intervals of 100*u* were cut using a Jung motorised sledge microtome. These sections were then stained by Haemotoxylin and Eosin and examined by light microscopy on 3 occasions by the author and Dr. M.A.M. Rozenbilds, Dept. of Pathology, F.M.C. At the time of interpretation of the material the operation details were not known to the observers. Observations were made of the state of the epiphyseal plate and the presence of bone bridges, or of bone fusion between the epiphysis and metaphysis. A fusion was determined as being present or absent.

The host tissue response of the epiphysis, metaphysis, periosteum, and physis was determined.

The normal physis shows the relative proportions of the resting, proliferative, hypertrophic and mineralizing zones of the sheep (See Fig. 3.6.2, Chapter 3).

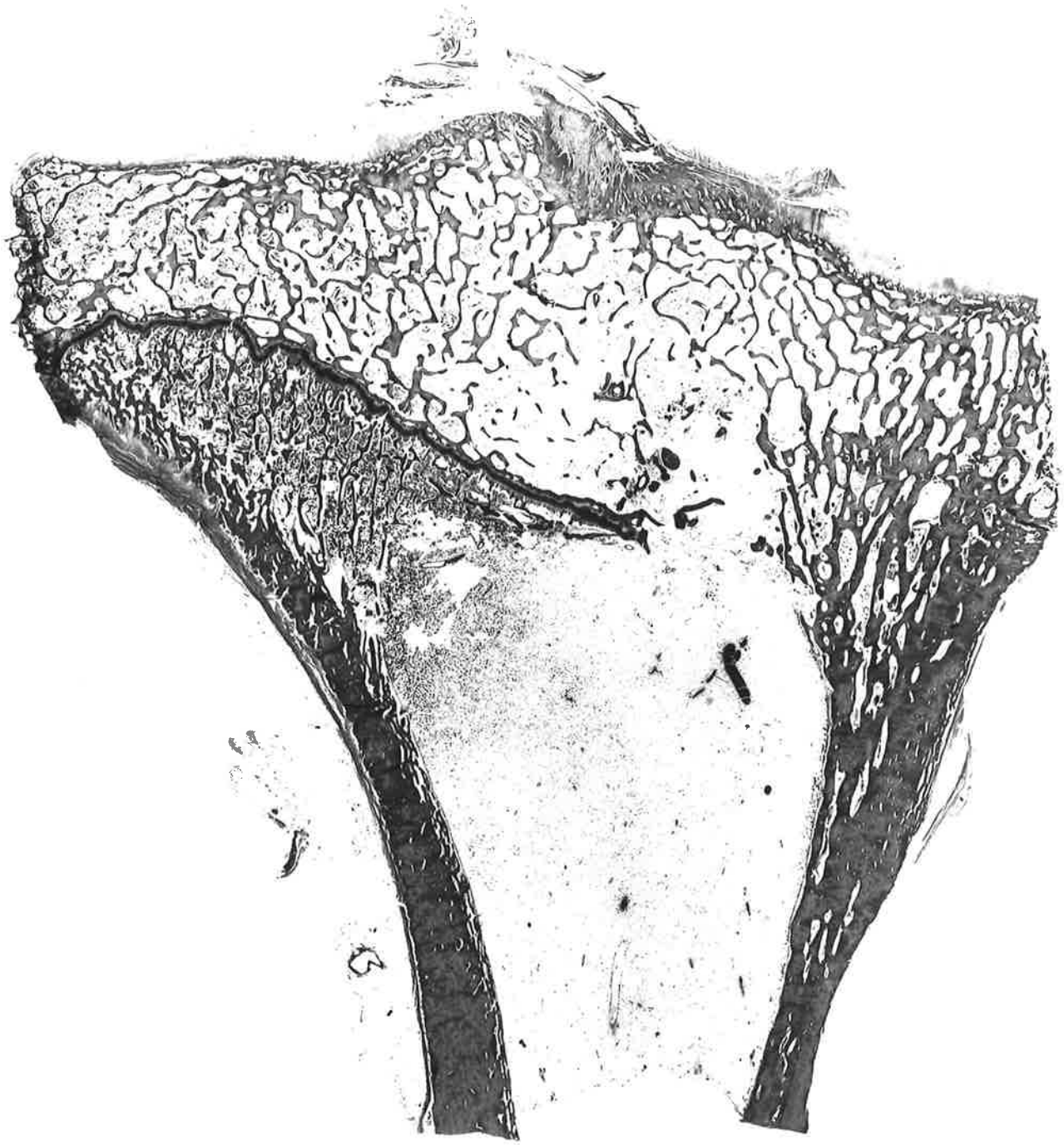
In these large specimens the angular deformity can be seen (Fig. 4.9).

4.4 RESULTS:

Complete data was obtained from 8 animals with 1 sq. cm. defect, and 2 animals with 2 sq. cm. defect, created in the physis after the techniques of Phemister (1933).

Figure 4.9: MACROSCOPIC VIEW OF A PROXIMAL TIBIAL FUSION.

Deformity of the bone is apparent with varus angulation. A peripheral cortico-cancellous complete bar is seen joining the epiphysis and metaphysis. (x 4)



4.4.1 Radiological Results:

The results for the radiological deformity index are listed in Table 4A.

There was marked variation in the degree of angulation deformity ranging from 4° valgus to 23° varus. The bone length ratio was directly comparable to the degree of varus. If the ratio was less than 0.800 after three months then complete radiological fusion had occurred. This was the case in 5 of 10 animals. With three animals the bone length ratio was greater than 0.800 but still less than 1.000, and these animals radiologically showed a partial fusion. Two animals failed to fuse and had stimulation of growth.

Fig. 4.10 shows the variability of the provocative procedure in producing a growth arrest.

4.4.2 C.T. Results:

The results of C.T. scan evaluation of percentage of the physis damaged by the creation of defects are shown in Table 4B. The Arithmetic Mean of the series was 24.2%. There was a wide range of defect sizes from 12.5% to 49.3% of the whole of the physis.

4.4.3 Bone Measurements:

The results of direct measurements of bone length and pin distance at autopsy are summarized in Table 4C.

4.4.4 Histology Results:

The histological occurrence of fusion and its correlation with the radiological deformity index are shown in Table 4D. There was fusion

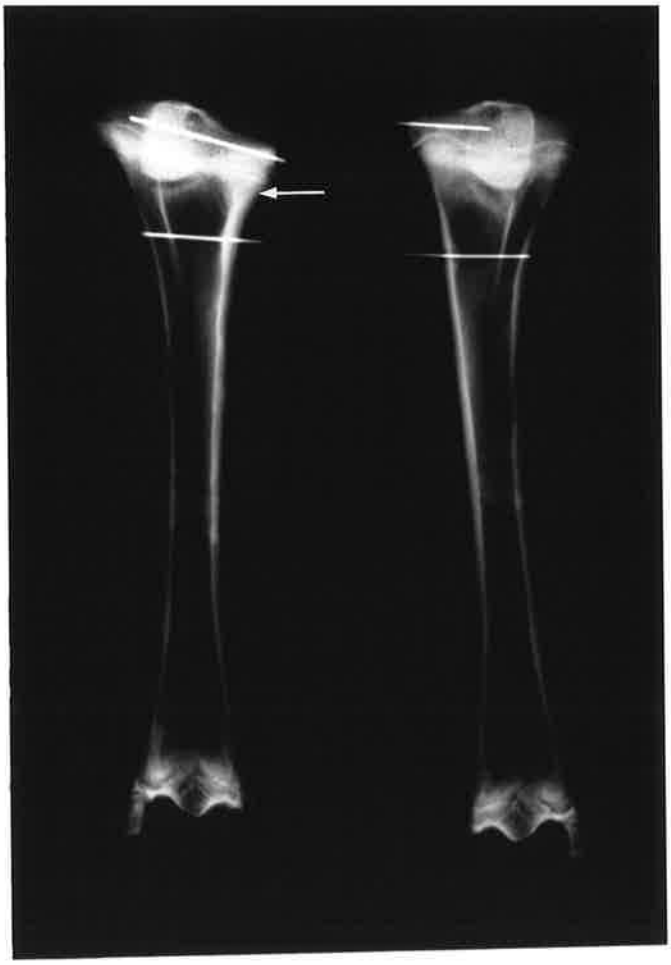
Figure 4.10: RADIOLOGICAL VARIATION OF FUSION.

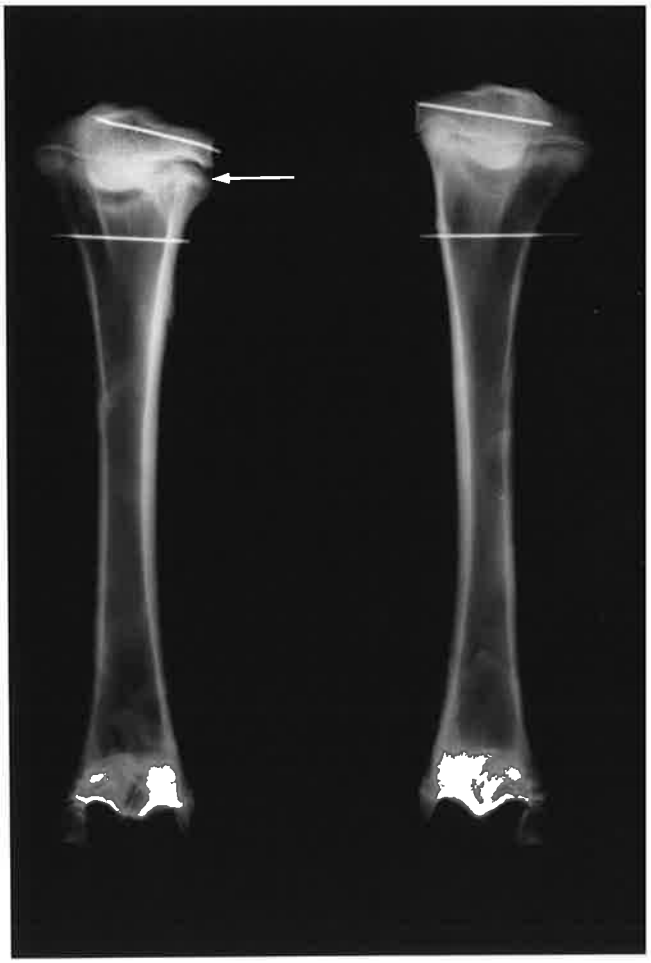
Figure 4.10.1: Fusion of the left tibia with varus angulation and shortening. (Animal 1)

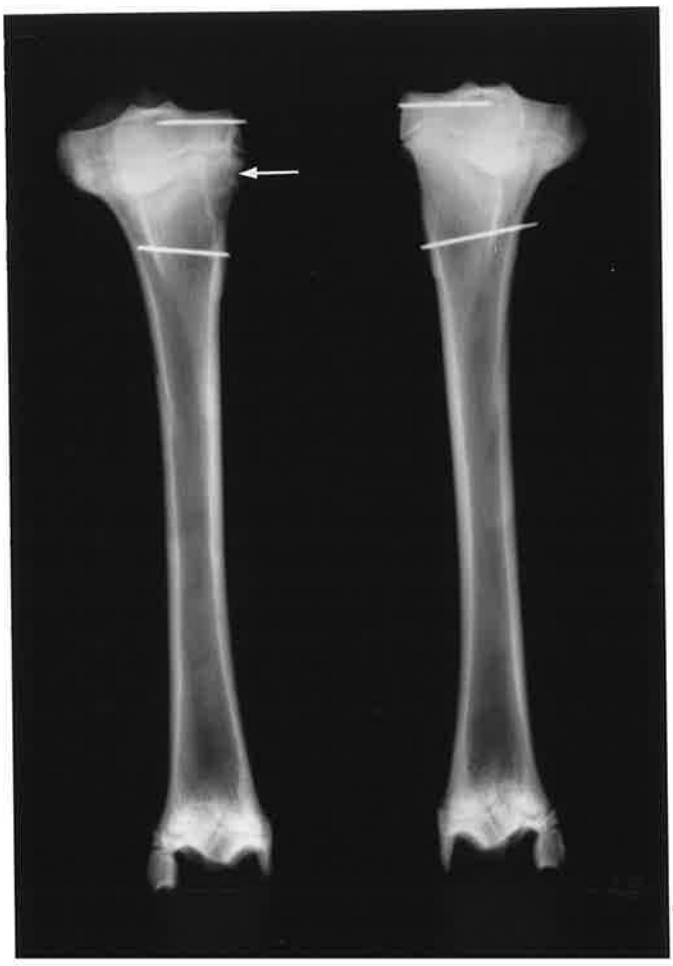
Figure 4.10.2: Partial fusion with an apparent widening of the physis, however angulation occurred. (Animal 4)

Figure 4.10.3: Non-fusion with continued growth. No apparent physeal bar medially. (Animal 3)

Figure 4.10.4: Non-fusion with apparent growth and overgrowth of the operated bone. (Animal 2)







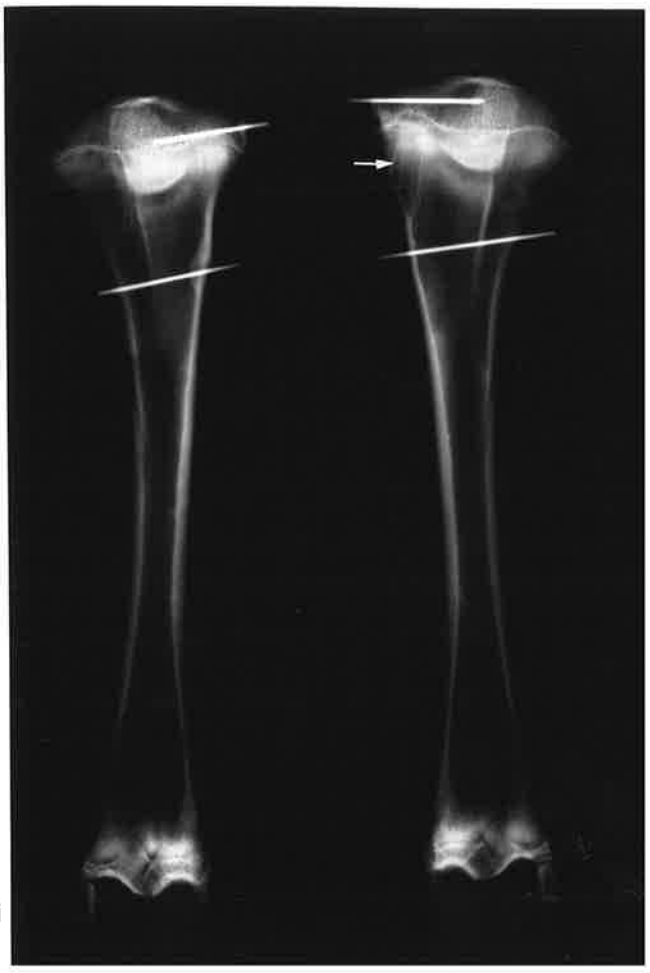


TABLE 4A

PROVOCATION SERIES

DEFORMITY INDEX AT 3 MONTHS

(i) Bone Lengthening Ratio

(ii) Pin angulation

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	($^{A}/_{B}$ ratio)	(at 3 months)	
1	6 18	0.135 0.139	0.142 0.201	0.950 0.692	18° varus	FUSION
2	6 18	0.132 0.200	0.143 0.203	0.920 0.985	4° valgus	NO FUSION
3	6 18	0.139 0.199	0.139 0.199	1.000 1.000	1° valgus	NO FUSION
4	6 18	0.139 0.192	0.139 0.219	1.000 0.877	10° varus	PARTIAL FUSION
5	6 18	0.134 0.136	0.134 0.190	1.000 0.716	13° varus	FUSION
6	6 18	0.140 0.193	0.142 0.218	0.986 0.885	9° varus	PARTIAL FUSION
7	6 18	0.145 0.113	0.148 0.212	0.980 0.533	23° varus	FUSION
8	6 18	0.140 0.195	0.140 0.210	1.000 0.928	6° varus	PARTIAL FUSION
9	6 18	0.144 0.118	0.149 0.201	0.966 0.587	13° varus	FUSION
10	6 18	0.146 0.122	0.147 0.207	0.993 0.589	22° varus	FUSION

C.T. SCAN RESULTS

TABLE 4B

Animal No.	Defect Total Area / Growth Plate Area Run 1	%	Defect Total Area / Growth Plate Area Run 2	%	Defect Total Area / Growth Plate Area Run 3	%	Average % Defect
1	$14.8/77.9$	19.0	$15.3/83.2$	18.4	$14.9/86.6$	17.2	18.2
2	$30.0/103.6$	29.0	$25.2/104.8$	24.1	$24.9/109.6$	22.7	25.3
3	$12.3/87.8$	14.0	$10.4/87.0$	12.0	$9.9/86.5$	11.4	12.5
4	$10.9/62.9$	17.3	$13.8/64.2$	21.5	$13.5/84.4$	21.0	19.9
5	$18.7/70.8$	26.4	$19.8/72.5$	27.3	$18.0/73.0$	24.7	26.1
6	$29.7/58.6$	50.7	$58.2/28.5$	49.0	$27.8/57.5$	48.3	49.3
7	$17.2/69.9$	24.6	$18.3/72.2$	25.4	$18.1/69.9$	25.9	25.3
8	$16.2/67.0$	24.2	$15.6/66.6$	23.4	$14.9/71.0$	21.0	22.9
9	$21.0/85.9$	24.4	$20.8/85.6$	24.3	$19.2/83.8$	22.9	23.9
10	$17.2/92.3$	18.6	$17.6/93.9$	18.7	$19.6/95.7$	20.5	19.3

TABLE 4C

DIRECT MEASUREMENTS AT SACRIFICE OF:

Bone Pin Distance Difference

versus

Bone Length Difference.

(at 3 months sacrifice)

Animal No.	Pin Difference (Short)	Comment	Bone Difference (Short)	Discrepancy
1	10 mm	Fusion	6 mm	4 mm
2	4 mm	No Fusion	1 mm (longer)	5 mm
3	0 mm	No Fusion	0 mm	0 mm
4	6 mm	Partial Fusion	5 mm	1 mm
5	12 mm	Fusion	10 mm	2 mm
6	5 mm	Partial Fusion	4 mm	1 mm
7	18 mm	Fusion	6 mm	12 mm
8	3 mm	Partial Fusion	3 mm	0 mm
9	15 mm	Fusion	10 mm	5 mm
10	13 mm	Fusion	10 mm	3 mm

in 4 animals by histological criteria and failure of fusion by radiological criteria in 6 animals.

Histologically, fusion has a characteristic pattern. At three months from the initial surgery a cancellous and/or cortical bar of bone extends as continuous trabeculae between the metaphysis and the epiphysis (Fig. 4.11.1). At higher power (x 150 magnification) the bone bar shows mature cancellous bone (Fig. 4.11.2).

The tether to the physis shows complete lack of organization by alteration of the width of the physis adjacent to the fusion mass (Fig. 4.12.1). Adjacent to the degenerative zone of the physis there appears to be an increase in the width of the zone of the proliferative and hypertrophic cells, although mineralization of the hypertrophic zone is normal (Fig. 4.12.2).

As noted by Harris, Martin & Tile (1965), the growth plate in the transferred segment may remain viable although functionless in the reversed bone block segment (Fig. 4.13). Where the periosteum has been disturbed the peripheral zone of Ranvier shows a disorganized physeal cartilage.

Non-fusion has a variable pattern. On inspection the bone is not apparently angulated or shortened. X-rays fail to show deformity or a bone bar across the physis.

Histologically there is fibrous tissue in the area of the bone bar defect. This is demonstrated in Fig. 4.14.1, 4.14.2.

Attempts at an incomplete bone bridge occur, and there may be a variable degree of tether. Immature woven bone creates the appearance of callus repair (Fig. 4.15).

Figure 4.11: HISTOLOGICAL EVIDENCE OF FUSION: BONE RESPONSE.

Figure 4.11.1: Animal 5 shows a peripheral bone fusion at the level of the Phemister procedure. (x 2.5)

Figure 4.11.2: The high power field shows a continuous bone bridge of cancellous bone. (x 150)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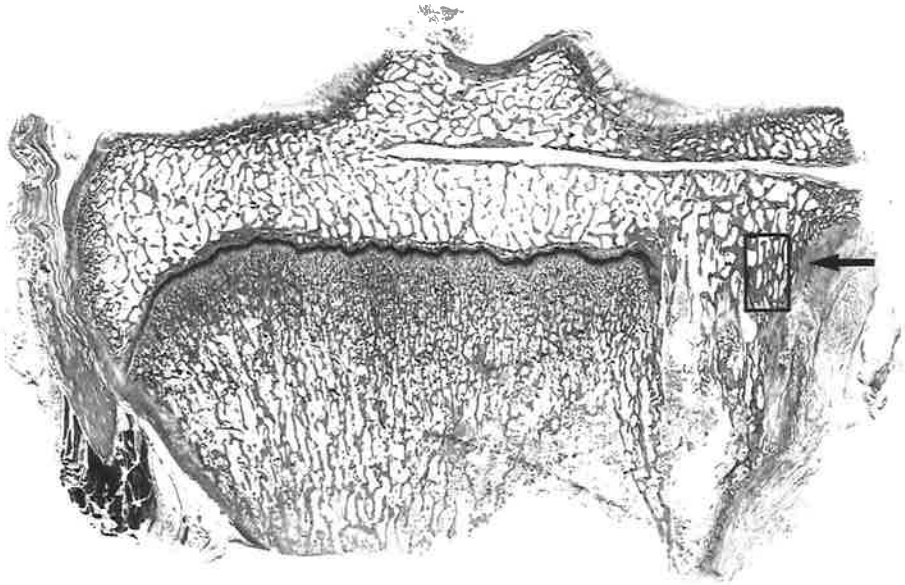
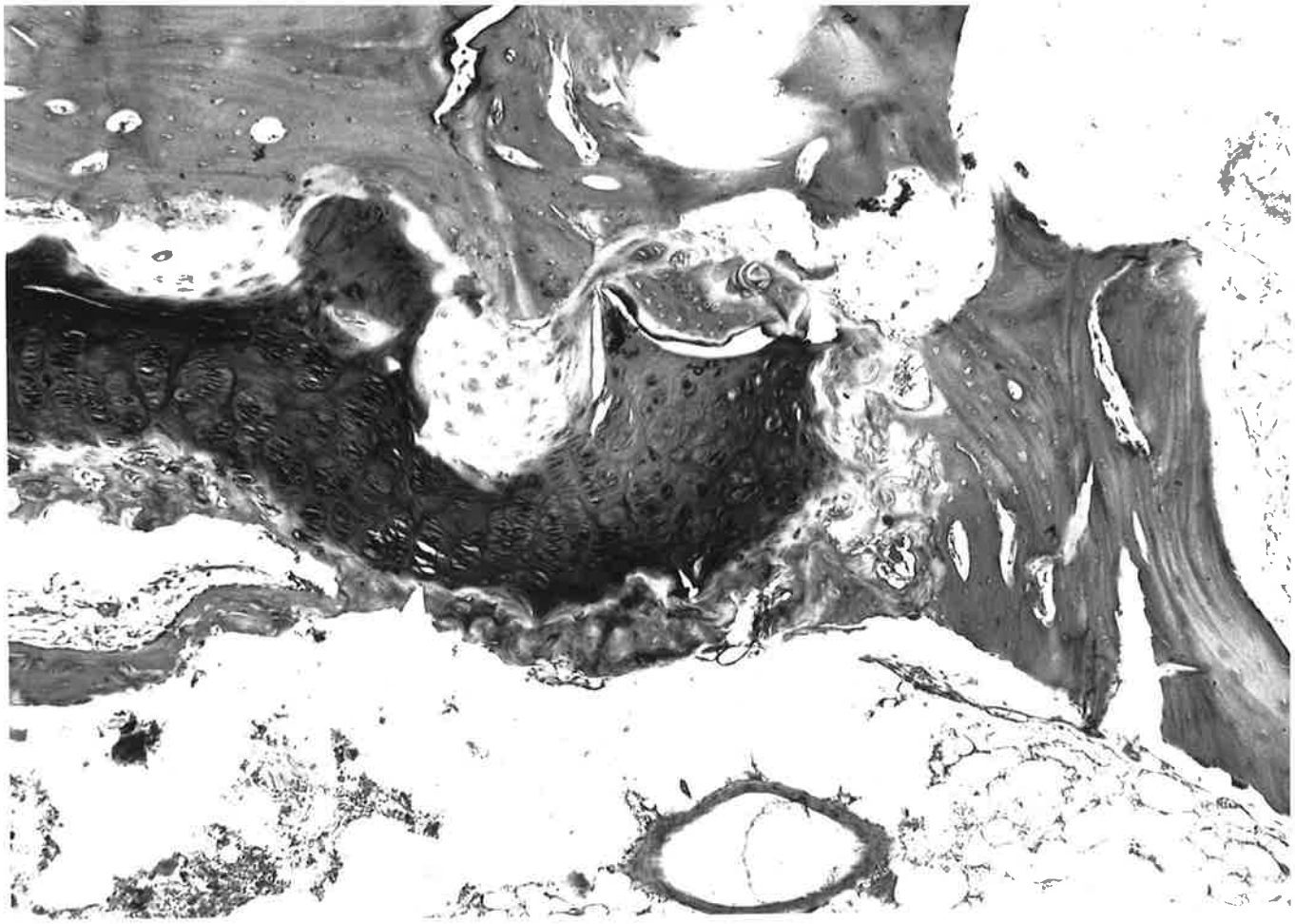


Figure 4.12: HISTOLOGICAL EVIDENCE OF FUSION: PHYSEAL RESPONSE.

Figure 4.12.1: Continuous bone tether immediately adjacent to an abnormal physis. There is loss of organization of the chondrocyte columns and the physis is narrower. (x 250)

Figure 4.12.2: Adjacent to the degenerative physeal zone there appears to be an increase in width of the prehypertrophic and hypertrophic cells. Mineralization is normal (x 250)



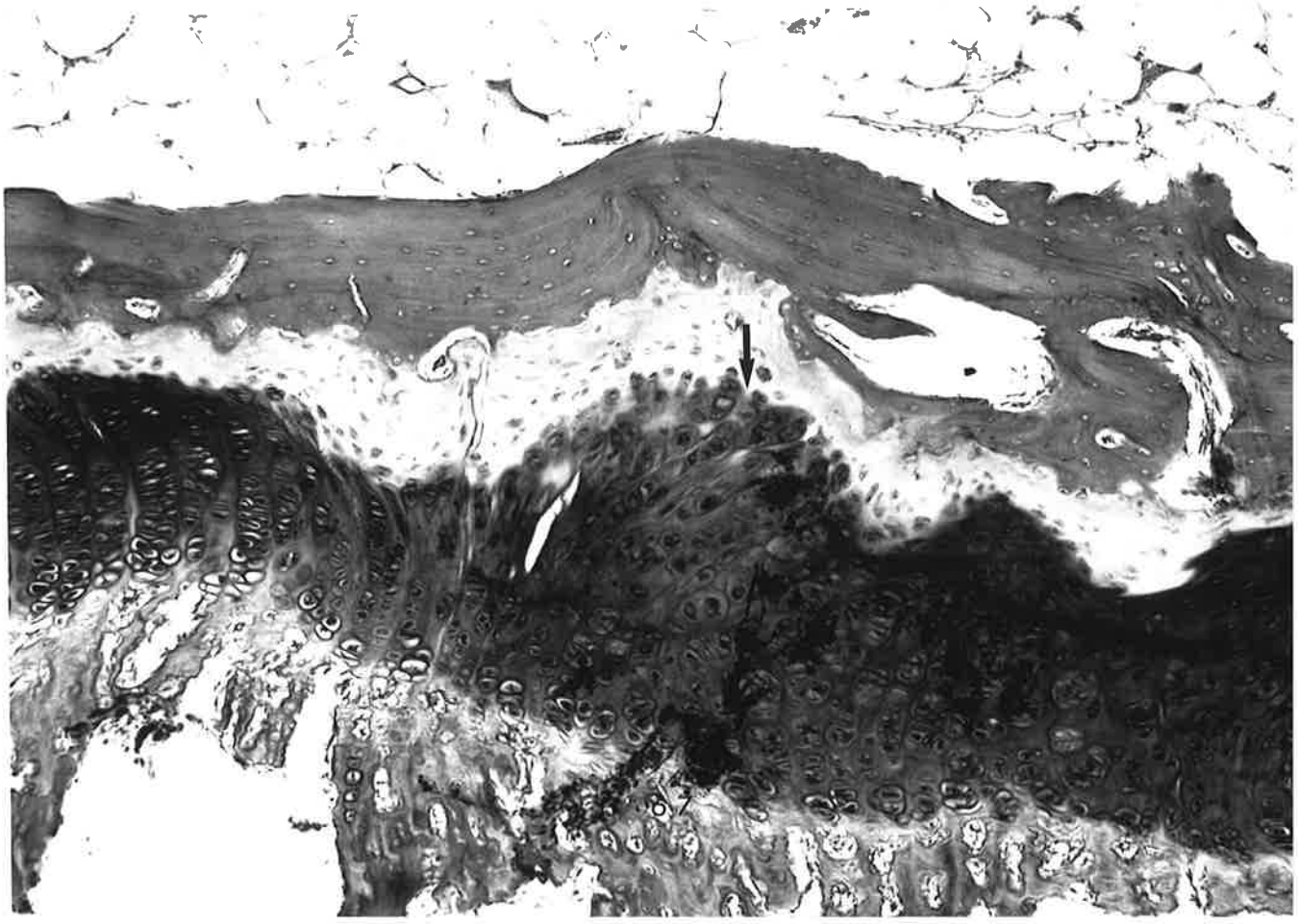


Figure 4.13: HISTOLOGICAL EVIDENCE OF NON-FUSION: PHYSEAL TRANSPLANTATION.

In the reversed bone block the physis can be observed to be present. ➡ It is disorganized and functionless at 3 months from the initial procedure. (x 4.5)

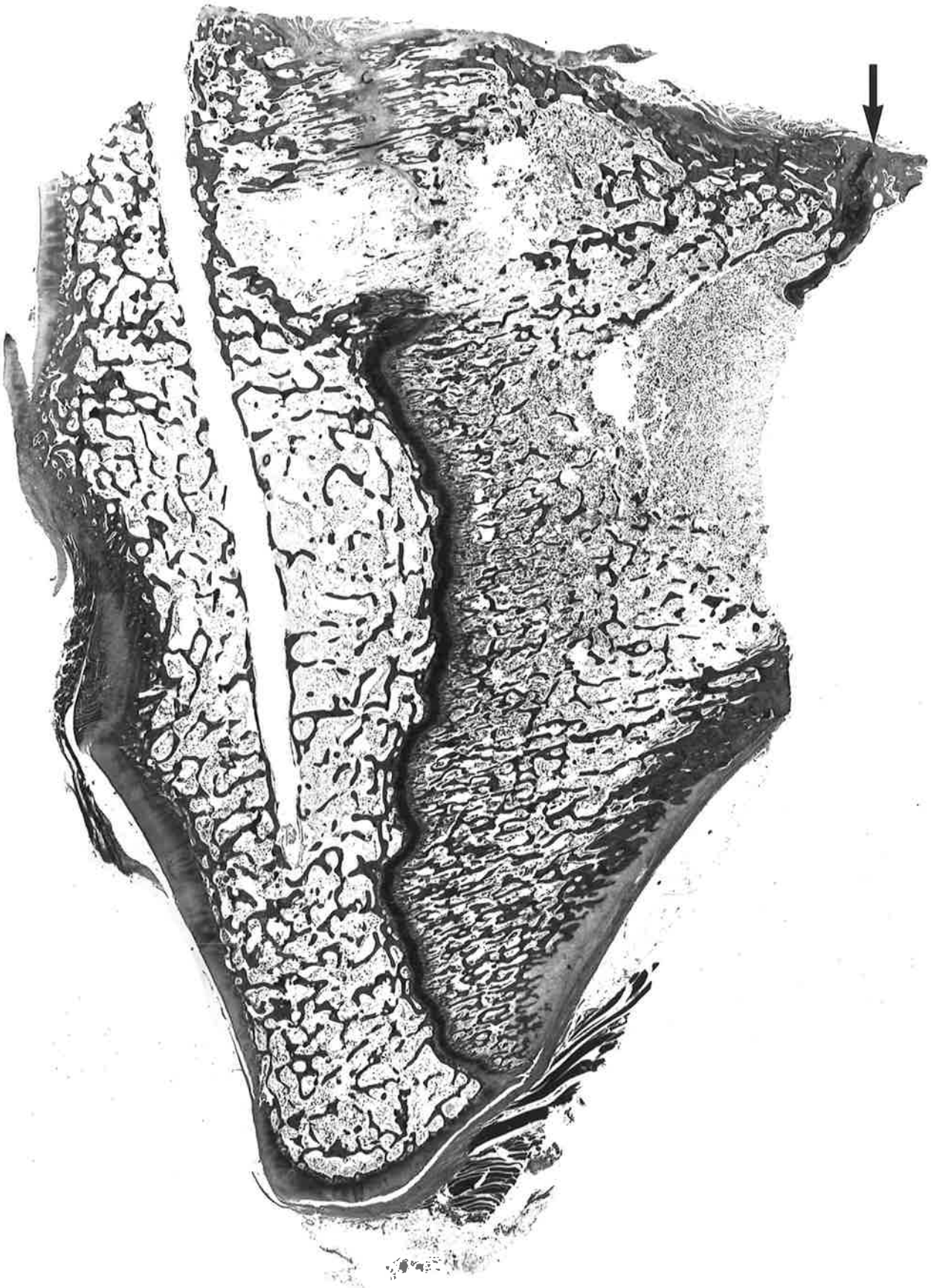
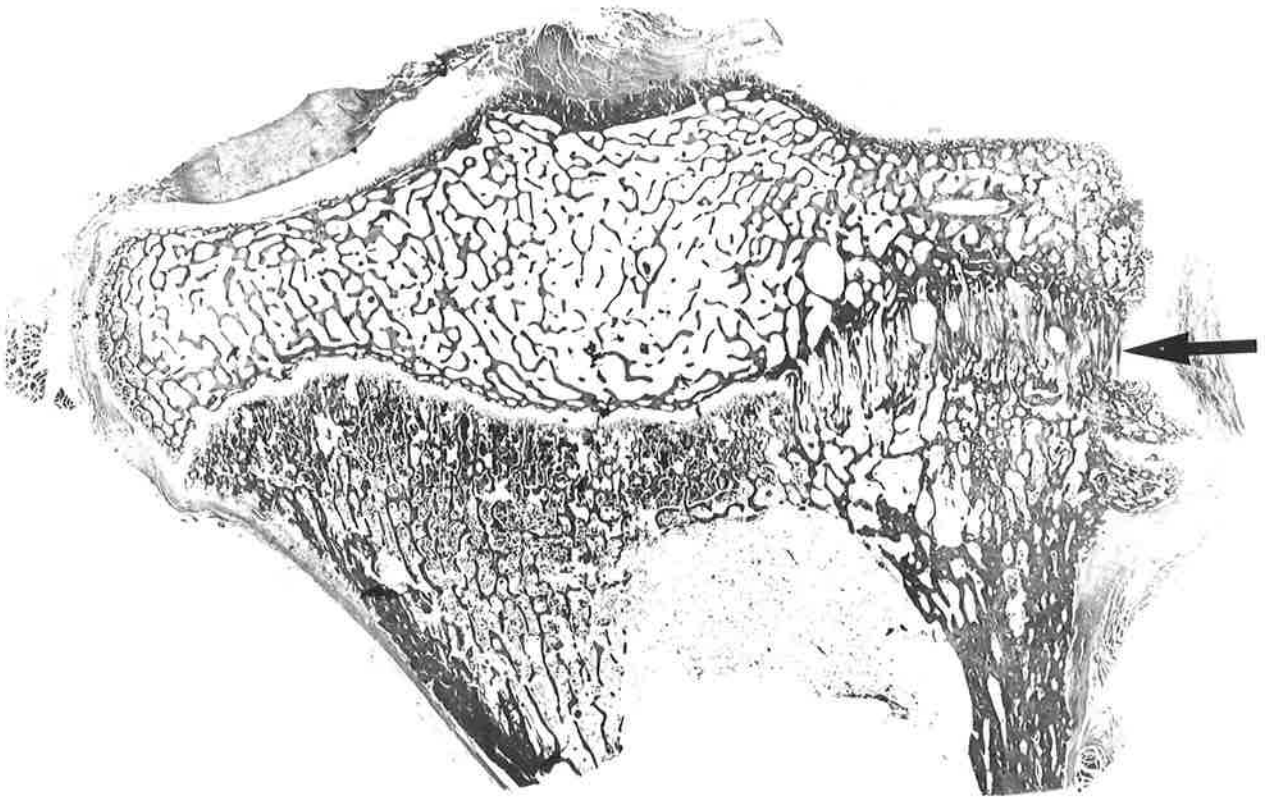


Figure 4.14: HISTOLOGICAL EVIDENCE OF NON-FUSION.

Figure 4.14.1: In Animal 2 where there was partial fusion the whole section of the proximal tibia shows the fibrous tissue and also peripheral bone physis proliferation at the level of attempted fusion. There is discontinuity of the bone trabeculae. (x 3.5) ➡

Figure 4.14.2: In Animal 8 the fibrous tissue shows more marked longitudinal discontinuity at the level of the attempt at physiodesis. (x 3.5) ➡



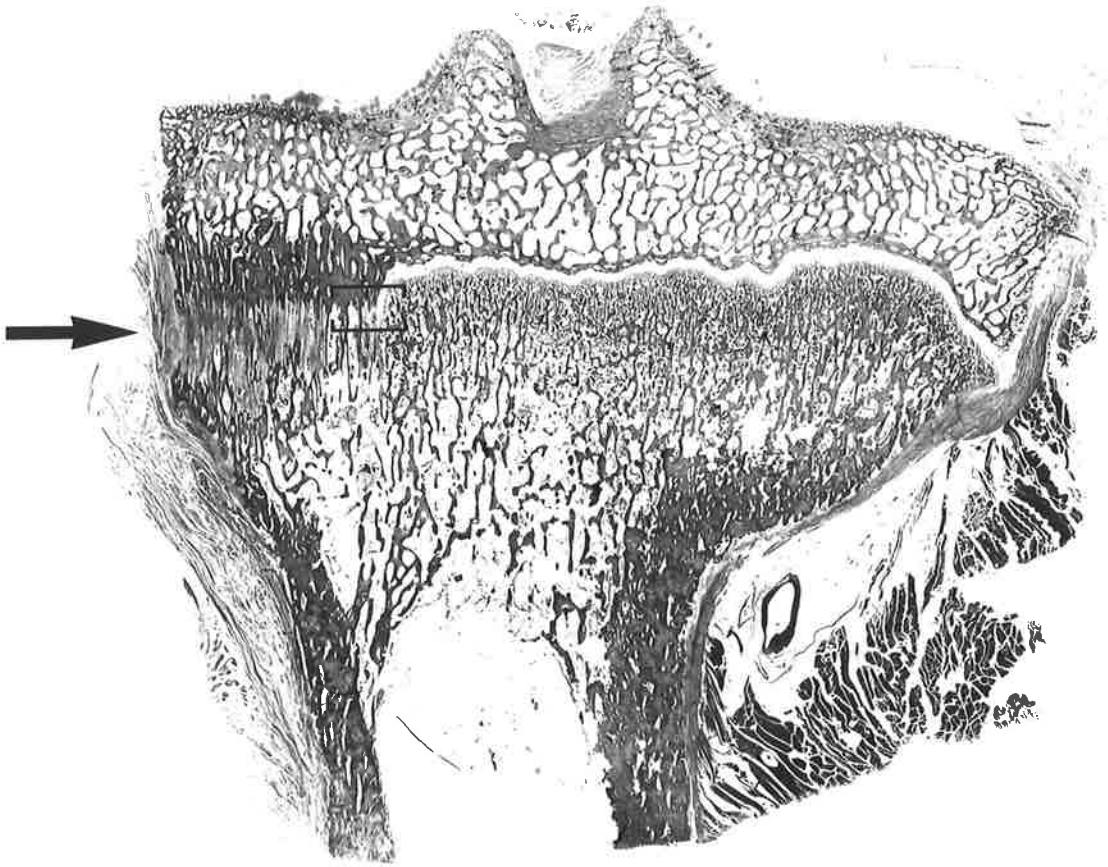


Figure 4.15: **HISTOLOGICAL EVIDENCE OF NON-FUSION. (Animal 8) (x 350)**

The fibrous tissue shows a longitudinal array with this being oriented along the lines of growth.

Immature cancellous bone shows a fracture effect at the level of the former physis with discontinuity of the bone trabeculae.

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The fibrous tissue is longitudinally oriented, as are the spicules of bone of the operated segment. The physis shows angulation towards the metaphysis in the operated segment, and this edge of physis is further disorganized. The width of the proliferative zone is increased and shows lack of longitudinal array. The proliferative zone cells are more cellular and also show increase of intercellular matrix.

4.5 DISCUSSION:

The histological findings of fusion and radiological parameters of fusion do not demonstrate an absolute correlation (Table 4D).

TABLE 4D

CORRELATION OF RADIOLOGICAL

& HISTOLOGICAL FUSION

Animal No.	Radiological Result	Histological Result
1	Fusion	No Fusion
2	No Fusion	No Fusion
3	No Fusion	No Fusion
4	Partial Fusion	No Fusion
5	Fusion	Fusion
6	Partial Fusion	No Fusion
7	Fusion	Fusion
8	Partial Fusion	No Fusion
9	Fusion	Fusion
10	Fusion	Fusion

For example, the first animal had a radiological fusion that was not confirmed histologically. This exemplifies the difficulty of quantification of the results of this series of experiments.

From Table 4A radiologically the result was a varus deformity of 18° and a decrease in bone length ratio from 0.950 to 0.692. From Table

4C there was a 10 mm. of length discrepancy between the pins, but only a 6 mm. overall bone length discrepancy.

The overall length discrepancy from physeal damage is less than predicted. This is true in all the experiments. The second experimental animal had one leg being 1 mm. longer than the control. This suggests that the distal tibial epiphysis may contribute to a "catch-up" phenomenon of growth to the length of the whole bone as described by Heikel in 1961. This particular result, in conjunction with the occurrences of non-fusion both radiologically and histologically in other cases, confirms that there is a variable degree of damage to the periphery of the growth plate that can be withstood without producing deformity.

Friedenberg in 1957 commented upon this phenomenon and suggested that the ability of the physis to respond to minor peripheral deformities induced micro fractures of the osseous bridges. Campbell, Grisolia & Zanconato (1959) and Johnson and Southwick (1960) confirmed these findings. The results of the experiments described confirm these findings although question the interpretation of the histological result since it is more probable that a bone bridge does not ever form and hence cannot fracture. The immature fibrous tissue longitudinally arrayed in the defect between the epiphysis and the metaphysis represent immature callus.

The C.T. scan (Table 4B) gave a measure of the extent of physeal damage and showed this could be used to estimate the extent of undamaged physis.

The density of tissue cavities recorded in Hounsfield Units is such that the density of fat is from 0 to (minus) - 150°. In the extremities no tissue other than fat has a negative H.U. density

value. Any fluid that accumulates in bones has a positive value. Thus adipose tissue can be identified in extremities with great accuracy.

To monitor the change in the tissue cavities with fat interposed as a graft the repeated scan would thus give information on the change in the size of the growth plate defect.

There was a wide variation (Table 4B) (12.5-49.3%) in the percentage damage to the physis produced by a standard 1 sq. cm. defect. This was thought to be due to the difficulty in estimating bone density difference (H.U.) between normal cancellous bone and the fibrous and bone tissue of the defect site. The contour of the physeal plate is not planar and so the scan will not constantly pass through the level of the normal physis. This would tend to over-estimate the damaged segment. The data suggests that with small areas of damage, for example in animal 3 where the size of defect was 12.5%, the physis can continue to function and even break down bone bridges or prevent their formation by a micro fracture effect. In the intermediate range of deficiency of 18.2%-26.1% a fusion may or may not occur. This is determined by biological variants such as whether the periosteum forms a bone bar that does not fracture, or whether the peripheral periosteal, pluripotential cells repair the physis sufficiently to maintain physeal function.

The C.T. estimation of 49.3% of physeal defect in animal number 6, with partial radiological deformity not confirmed by bone bridge formation, histologically suggests that the primary problem is the dysfunction of the physis itself rather than a bone bridge preventing its function. Alternatively, the higher percentage could represent a gross over-estimation of the defect size, and is of concern when

looking at determining the extent of the defect which could be filled with fat.

Fat is the only tissue on the extremities to have negative H.U. and thus represents another marker to determine the extent of the physis resected at reversal operations. It also enables serial C.T. measurements to determine with time the size of the defect.

4.6 CONCLUSION:

These experiments confirmed that the technique of Phemister was appropriate to a sheep tibia model to produce partial peripheral physeal dysfunction, but that this peripheral lesion to the physis may not cause a premature growth arrest.

Radiologically, a deformity index of fusion can be a reliable indicator of the formation of a bone bridge across the physis. However the shortening and deformity may also be a result of the extent of the growth of the physis that has been compromised.

Animals not showing radiological evidence of angular deformity and shortening would be discarded from the reversal operations.

The next series of experiments evaluate the reparative potential of the physis, determine the extent to which an interpositional material could prevent bone bridge formation, and show the mechanism(s) of the recovery of physeal function.

Chapter 5 FAT INTERPOSITIONAL REVERSAL

5.1 EXPERIMENTAL AIMS:

A series of experiments was undertaken to determine:

(i) the fate of free fat graft implants

(ii) the extent of peripheral physis that can be resected and replaced with free fat to restore normal growth

(iii) the reparative potential of the physis.

5.2 REVIEW OF THE LITERATURE:

The treatment of choice in injury of the physis that causes deformity has generally been a corrective osteotomy. As growth continues, however, the deformity recurs, and thus the osteotomy may need to be repeated. Alternatively, in addition to an osteotomy the rest of the physis can be destroyed to prevent recurrence of angulation. This strategy results in the loss of longitudinal growth of the bone, and thus in limb shortening.

In other treatments to equalize leg length the opposite leg may require shortening by either epiphyseal plate arrest (physiodesis) or by a shortening operation at skeletal maturity [Phemister 1933, Eyre-Brook 1951, Green & Anderson 1955, Menelaus 1966, Bianco 1978, Winquist 1978]. A bone lengthening operation on the shortened leg is also possible [Codivilla 1905, Magnuson 1913, Putti 1921, Abbott & Crego 1928, Compere 1936, Sofield 1939, Harmon & Krigsten 1940, Abbott & Gill 1942, Brockway & Fowler 1942, Phalen 1942, Allan 1948, McCarroll 1950, Anderson 1952, Blount 1954, Goff 1960, Salter & Harris 1963, Kawamura 1968, 1981, Ilizarov 1969, Wagner 1971, 1978,

Stephens 1978, Ray 1978, Moseley 1978, Monticelli & Spinelli 1981c, De Bastiani 1986]. There are complications associated with these techniques that include; pin tract infection, fractures and non-union of the lengthened segment, dislocation of joints [Coleman 1986, Moseley 1988].

Many investigators have performed animal experiments to prevent the progression of deformity due to physeal fusion by removing a portion of the physis and immediately replacing it with an interpositional material.

In 1878, Vogt was the first to investigate the effects of epiphyseal separation. In a goat he used gold leaf as interpositional material on the proximal tibia, and in a dog a rubber film on the distal radius. He was able to prevent the growth of capillaries between the epiphysis and the metaphysis.

In 1956 Ford and Key used gel foam in a study of experimental trauma to the distal femoral epiphysis in rabbits.

Also in 1956, Friedenberg and Brasheer used bone wax in the distal femur of the rabbit. Further results in the same model in 1957 were reported by Friedenberg who used bone wax and methyl methacrylate.

In 1958 Key and Ford used bone wax in the distal femur of the rabbit.

In 1970 Serafin used bone wax and muscle in the distal femur of the rabbit. The free muscle transplant was most effective in preventing bone bridge formation across the traumatized epiphyseal plate.

In 1983 Lennox, Goldner, and Sussman used fat and cartilage in a rabbit distal femur model. Their results suggested that cartilage was superior to fat as an interpositional material.

All these experiments have included a peripheral injury to the physis (a type VI injury) to create a peripheral defect. An interpositional material was used to prevent physeal bar formation, and any subsequent growth was observed. Results have been varied, but there has been sufficient success to suggest that bone bar formation can be inhibited or prevented.

When no interpositional material is used bone bar formation occurs consistently, [Österman 1972, Lennox, Goldner & Sussman 1983] although Bisgard (1937), noted difficulty in obtaining fusion in goats. Difficulty in obtaining fusion was also confirmed in Chapter 4.

In the clinical field there are no reports of interposition materials being used in human studies for the prevention of bone bridges at the time of acute traumatic physeal injury. The technique of fat interposition has been utilized by the author at the Adelaide Children's Hospital during the open reduction of cases of comminuted type IV fractures and type VI fractures. Although follow-up has not been to skeletal maturity, results have been encouraging, and in all cases prevention of bone bridging has occurred. Another application of this technique might occur during the excision of a benign tumour, a process which sometimes includes loss of the physis.

The treatment of an established physeal bar by excision of the bar has an experimental basis. The presence of injured or dead portions of cartilage in an epiphyseal plate has been

shown to prevent the formation of a physeal bar following injury [Langenskiöld & Edgren 1949, Heikel 1960].

The mechanism of repair of the injured portion of the physis has been assumed to be from regeneration of the adjacent parts of the growth plate by a process of lateral creep. Nordentoft in 1969 observed that the physeal cartilage regenerates and fills a drill hole transversing the epiphyseal plate. However, in experiments where a bony connection exists between the epiphysis and the metaphysis no regeneration has been observed [Ford and Key 1956, Friedenberg 1956, Campbell, Grisolia, Zanconato 1959].

Animal experiments have also been performed in which a bone bar is created then excised. Various interposition materials have been used with varying degrees of success.

Bone wax has been most often used [Key and Ford 1958, Campbell, Grisolia, Zanconato 1959, Serafin 1970]. In 1957 Friedenberg used bone wax in the distal femur in 10 rabbits, but the results were not encouraging because of fragmentation of the bone wax.

In 1969 Nordentoft used no interpositional material in the dog proximal tibia following epiphyseal/metaphyseal separation concurrent with removal of a bone bridge between the epiphysis and the metaphysis. He did not achieve positive results by this method.

In 1972 Österman reported the most comprehensive series of experiments using fat or cartilage or bone wax in the distal femur of rabbits. He concluded that suitable interpositional

materials prevent the recurrence of the bone bridge, and that deformity may be corrected. He suggested that cartilage may be the best material. Langenskiöld (1986) has also demonstrated the effectiveness of fat as an interpositional material in a pig model.

In 1974 Bright used silicone rubber, silicone adhesive, or isobutyl cyanoacrylate in the medial distal femur of dogs. His conclusions were that the silastic elastomer prevented bone bridge reformation.

Sudmann in 1982, used no interpositional material in the rabbit distal femur model of partial closure of the physis, and reported on the delay of bone bridge reformation with indomethacin. The mechanism of this is conjectural, and has been substantiated in work by Connolly et al (1988).

The first case in a human of fat interposition was reported in 1967 by Langenskiöld. The case involved a 15 year old boy with genu recurvatum, secondary to a bone bar in the anterior proximal tibia. The aetiology was unknown. The bone bar was excised in 1965 and the space filled with autogenous fat. During the 1.5 year follow up the angle of genu recurvatum improved 10 degrees, but there was no documentation of longitudinal growth.

The first case documenting longitudinal growth was reported in 1980 by Peterson. In this case a tibia grew 16.7 cms after excision of a distal tibial bar in 1968 in a boy who was then 5 years of age. Follow up continued for 10 years; sheet silastic and gel foam were used as the interpositional materials.

Multiple series of clinical cases have since been reported in which several different materials have been used:

(i) fat alone was reported by Langenskiöld (1967, 1975, 1978, 1979, 1981, 1986, 1987) and Visser and Nielsen (1981);

(i) fat and bone wax has been reported by Vickers (1980);

(iii) Silastic (Silastic Elastomer No. 382 - Dow Corning) has been reported by Bright (1978, 1982);

(iv) Methyl Methacrylate (Cranioplastic, [no barium] manufactured by L.D. Caulk Company, Milford, Delaware, U.S.A. 19963) has been reported by Mallet (1975, 1978,) Vickers (1980), Klassen and Peterson (1982).

These methods appear to be most popular. However, few cases have been followed to maturity to permit assessment of superiority of any one interpositional material.

The use of fat has the advantage of being autogenous, and no foreign material is inserted. Usually, enough fat can be found in the same incision to fill the defect, although sometimes other fat must be harvested from another site of the body such as the buttock. Langenskiöld (1975) recommends the use of buttock fat.

Fat, however, has the disadvantage of not producing haemostasis in the resected cavity. When the tourniquet is released the fat may float out of the cavity as bleeding occurs from the bone. Bone wax may be used to diminish the bone bleeding [Vickers 1980].

Closing the periosteum over the defect to contain the fat also predisposes to new bone formation peripherally, and to the reformation of a physeal bar. This is undesirable since it may tether growth again.

The operative defect also weakens the structure of the bone and therefore may predispose to pathological fracture [Vickers 1980, Visser & Nielsen 1981]. Gradual ossification of the metaphyseal defects do seem to occur [Peterson 1980]. The fate of the fat in the metaphyseal cavity is still undocumented. Langenskiöld (1987) suggests that hypertrophy and elongation of the autogenous fat occurs with progressive decrease in the size of defect in the resected physeal segment. This is reported from animal and clinical data [Langenskiöld, Videman, & Nevalainen 1986].

Of the other interpositional materials, silastic is inert, non-reactive, and virtually unaffected by long term exposure within the body. When mixed with a catalyst, silcone-rubber monomer vulcanizes. While it is in the semipolymerized state the silicone rubber can be moulded and pressed into the surgical field to fill the exact form of the cavity. It must remain in direct contact with the exposed physis to prevent bar reformation. Sterilization of both the monomer and the catalyst is effected 12 hours prior to surgery. During the operation a culture is taken of the silastic after mixing but before insertion. The implant is free-floating and easily removed later. Silastic is currently controlled in the U.S.A. by the Food and Drug Administration (F.D.A.) and its use requires authorization since it is an Investigational New Drug.

Bright (1982) recommends the use of Silastic elastomer No. 382 (Dow Corning).

Recommended by Klassen and Peterson in 1982, methyl methacrylate is light, thermally non-conductive, transparent, and easy to mould. Both the liquid (monomer) and the powder (polymer) are sterile as packaged, and may be mixed in the operating room. It is unnecessary to obtain cultures. It has had wide clinical trials in both neurosurgery and orthopaedic surgery. When used as an isolated substance it has caused no rejection, infection, or neoplastic change [Cabanela 1972].

Because the solid substance fills the cavity there is excellent haemostasis, and also structurally the bone integrity is maintained. Klassen and Peterson (1982) strongly recommend the use of methyl methacrylate without barium. "Cranioplast" has the advantage of being radiolucent, and this enables easier identification of physeal bridge reformation.

In 1979, Langenskiöld and Österman summarized the results of the first 33 patients who had undergone clinical osseous bridge resection with autogenous body fat implantation. In this series there were 22 males and 11 females with a mean age of 10.3 years at the time of surgery. Five patients were operated upon too recently for assessment and were not evaluated. Of the 28 patients whose results could be determined from the tabulated list of cases 15 appeared to have good or excellent results. This represents 54% of the cases. Five patients had fair results, equivalent to 18% of cases. Eight patients or 28% had poor results. Most of the poor results were due to

recurrent bridging, requiring repeat osteotomy or repeat resection of a recurrent osseous bridge.

In 1982, Klassen & Peterson reported at the Mayo Clinic (1968-1982) on 71 cases of bridge resection. "Cranioplast" was used in 68 cases, bone wax and fat in 2 cases, and silastic sheet and gel foam in 1 case. In all but one case there was some growth after bar excision. The growth of the bone operated upon as a percentage of the growth of the normal bone has varied from 0 - 200%, with a mean average of 94% [Klassen 1982]. For patients followed to maturity the average growth is 84% of normal. Thus, the renewed growth may diminish the angular deformity, and also the rate of progression of limb length inequality. Occasionally there may even be a reduction of the length inequality (the treated limb growing faster than the normal limb). In the one exception there was both proximal and distal tibial bar formation following previous irradiation for sarcoma of the fibula, there was no growth after bar excision at each site. Although the remaining physis appeared radiologically normal it may have had no potential for growth due to radiation damage.

In 1982, using Medical Elastomer #382 (renumbered as X7-2320 for human use) Bright reported on the first 100 of over 250 consecutive patients with at least a 3-year follow up from the date of surgery. 70% had good to excellent results, 12% fair results, and 18% poor results. Over one half of the poor results were children for whom the operation was performed too late. These children stopped growing within six months of the operative procedure, even though their bone age suggested at least one more year of growth remaining. For this reason the

recommendation was that a patient should have at least 2 years of remaining growth before considering physeal resection. Bright in 1982 also reported the occurrence of post operative infections that compromised a successful result.

The use of free fat transplantation is based on the observation that it remains fat tissue in its new location. In 1924 Lexer first discussed this phenomenon and it was confirmed in experiments by Österman in 1972 and Kiviluoto in 1976.

5.3 MATERIALS AND METHODS:

As previously described, after the method of Phemister, on the proximal tibia of 20 lambs at six weeks of age a 1 sq. cm. defect was performed. 15 lambs had 2 sq. cm. defects and 10 lambs had 3 sq. cm. defects. The contra lateral limb served as a control. It remained unoperated upon after the initial operation to place marker pins in situ.

X-rays were taken three months later to measure the bone deformity index. A confirmation of deformity was present in each case which was operated upon for the reversal procedure.

5.3.1 Bone Bridge Resection:

The bone tether to the physis was excised with a high speed dental drill until a healthy cartilage growth plate could be seen. The bone fragments were removed using irrigation to minimize heat damage to the physis (Fig. 5.1).

Fat was taken from the knee fat pad and placed in the defect as a free transfer after the method of Langenskiöld (1967). It

Figure 5:1: **OPERATIVE DETAILS OF PHYSEAL BRIDGE RESECTION AND FAT INTERPOSITION.**

Figure 5.1.1: Incision over the proximal tibia exposes periosteum after division of the soft tissue (held with the forcep).

Figure 5.1.2: Division of the periosteum between the marker pins. Once reflected there is a continuous bone bridge evident.

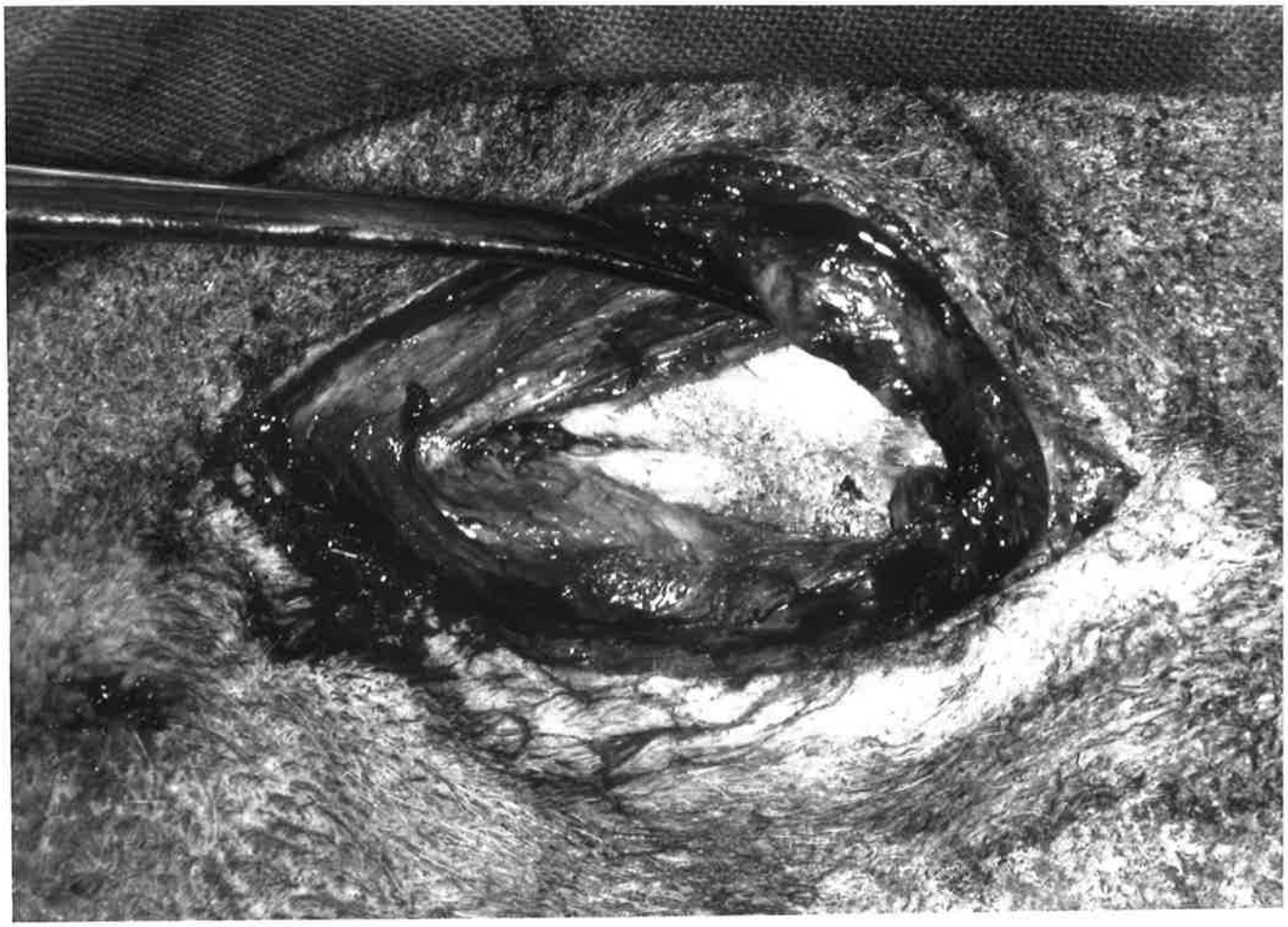


Figure 5.1.3: An osteotome removes the cortical bone below the proximal marker pin.

Figure 5.1.4: The bone shows the defect where the osteotome has cut the cortical bone.

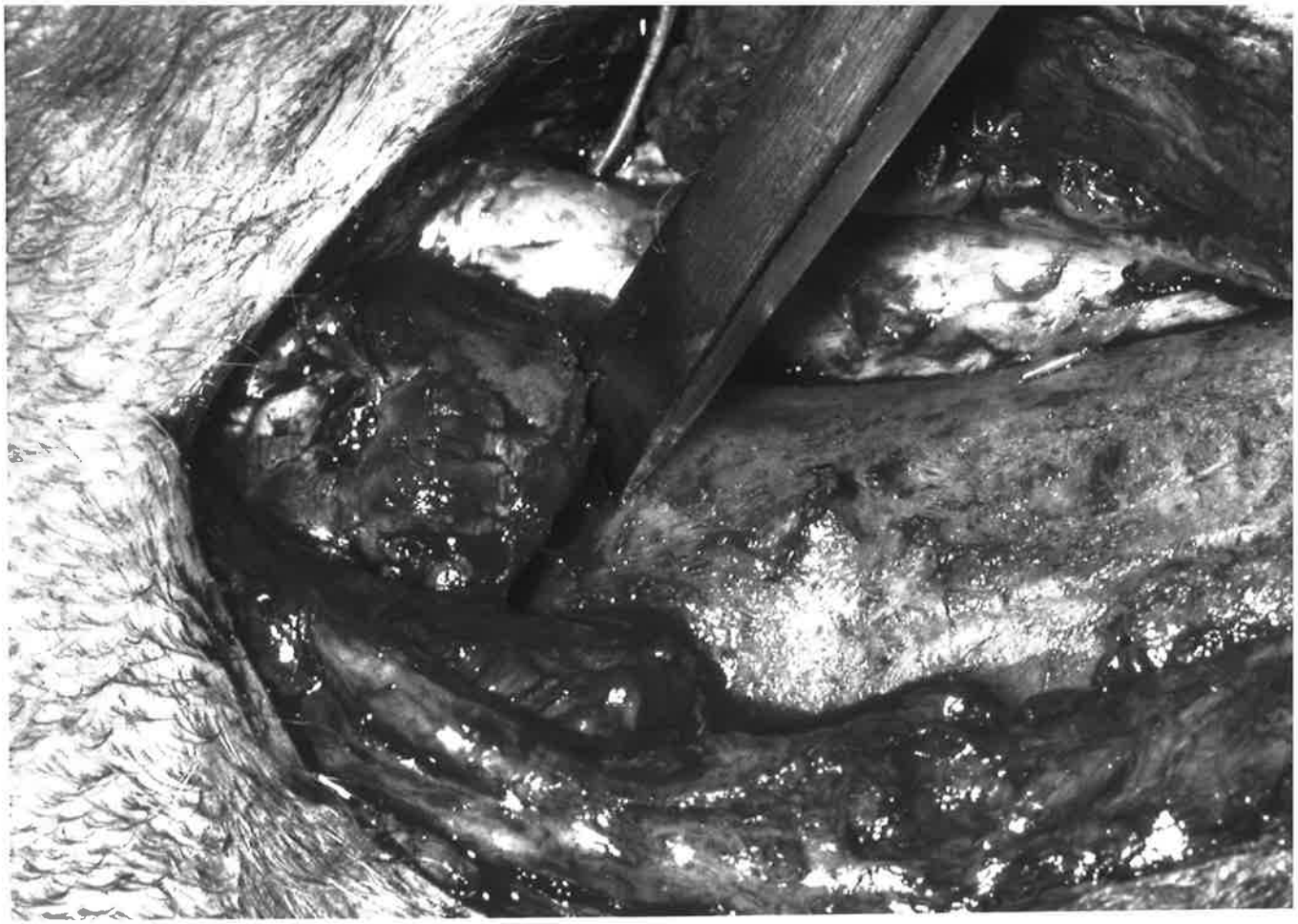


Figure 5.1.5: Removal of the cortical bone reveals the softer cancellous portion of the bone bridge.

Figure 5.1.6: The small 2 mm dental burr commences removal of the bridge. Binocular loupes (x 2.5) assist in identification of normal tissue.

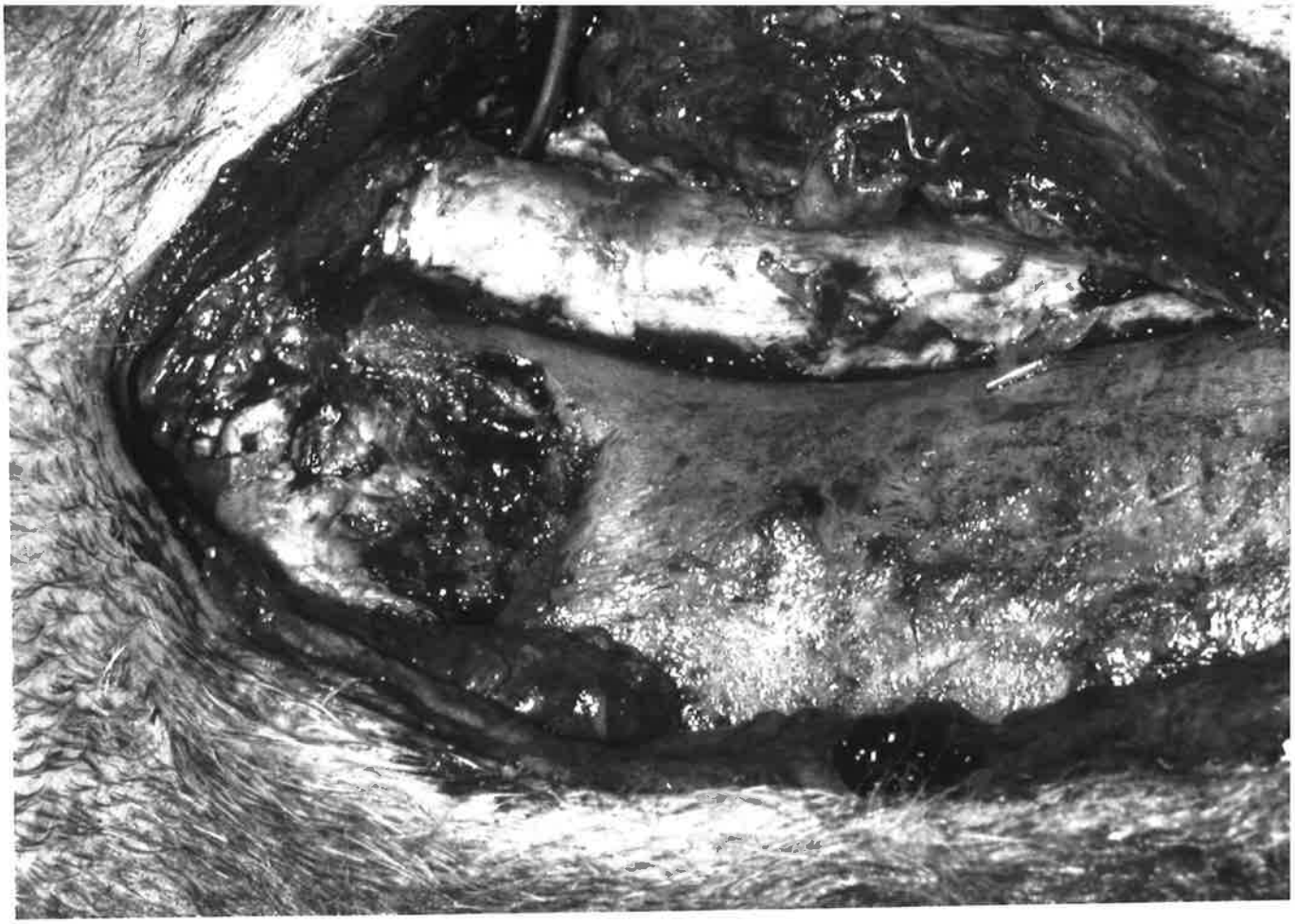
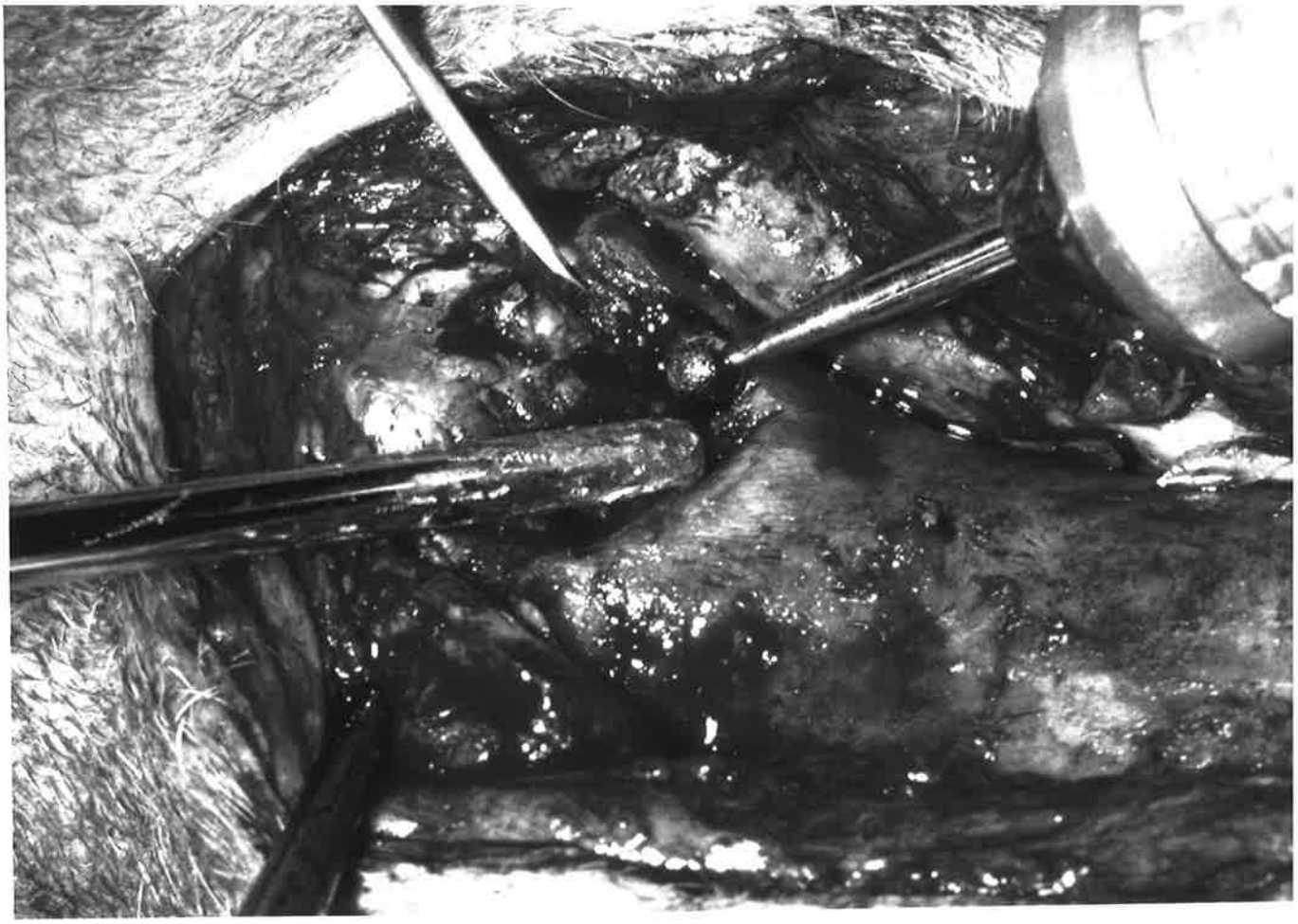


Figure 5.1.7: Irrigation with sterile saline through the needle diminishes soft tissue trauma in the operative field. The debris is removed with continuous controlled suction.

Figure 5.1.8: In the superior field of vision the normal residual physis can be seen. ➡

The defect in the epiphyseal metaphyseal junction is seen below the superior bone marker pin.



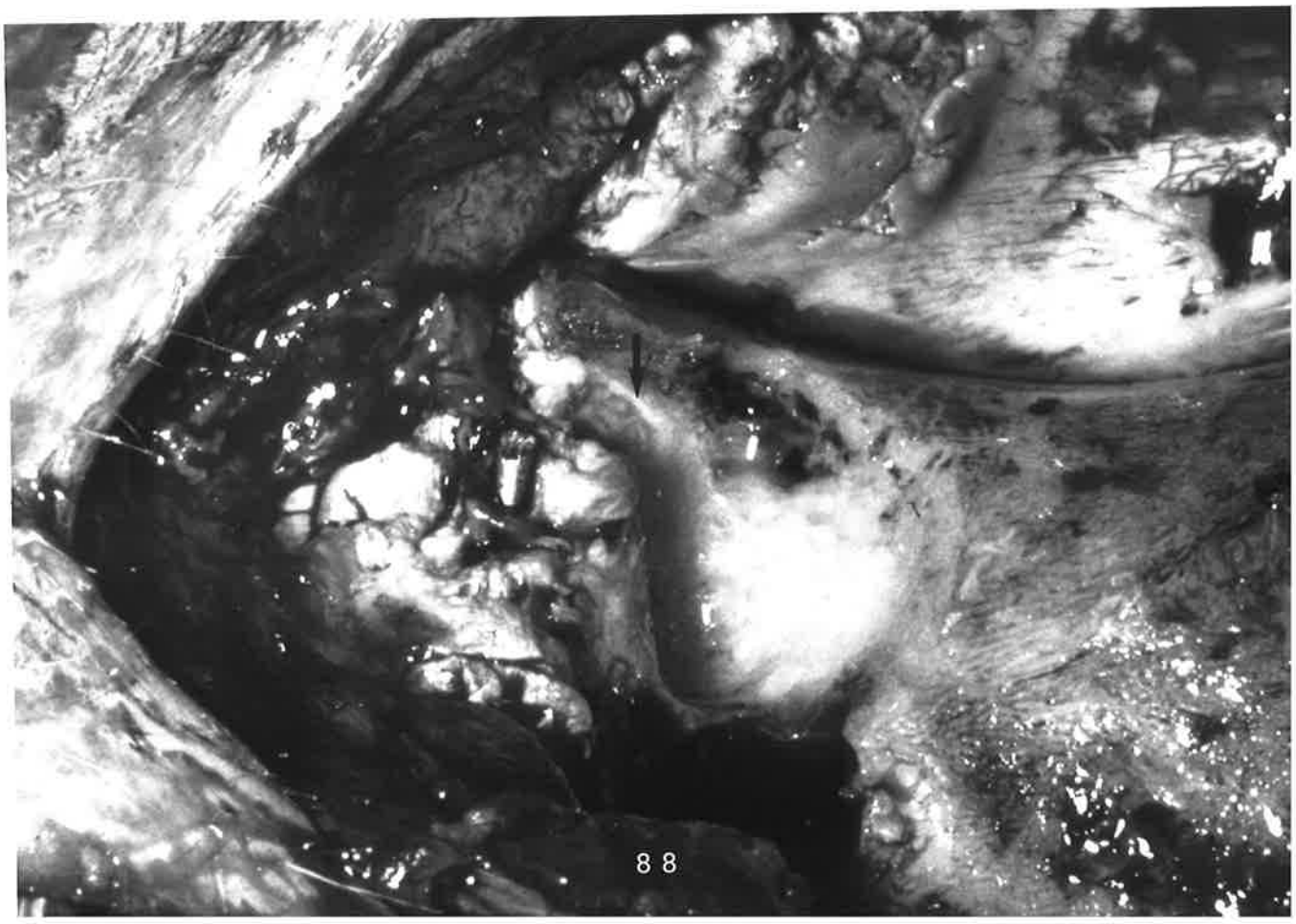


Figure 5.1.9: Showing the remnant physis superiorly and the dental burr progressively removing the bone bridge.

Figure 5.1.10: Following complete removal of the bone bridge the normal residual physis can be appreciated as a continuous line in the middle of the field of view.

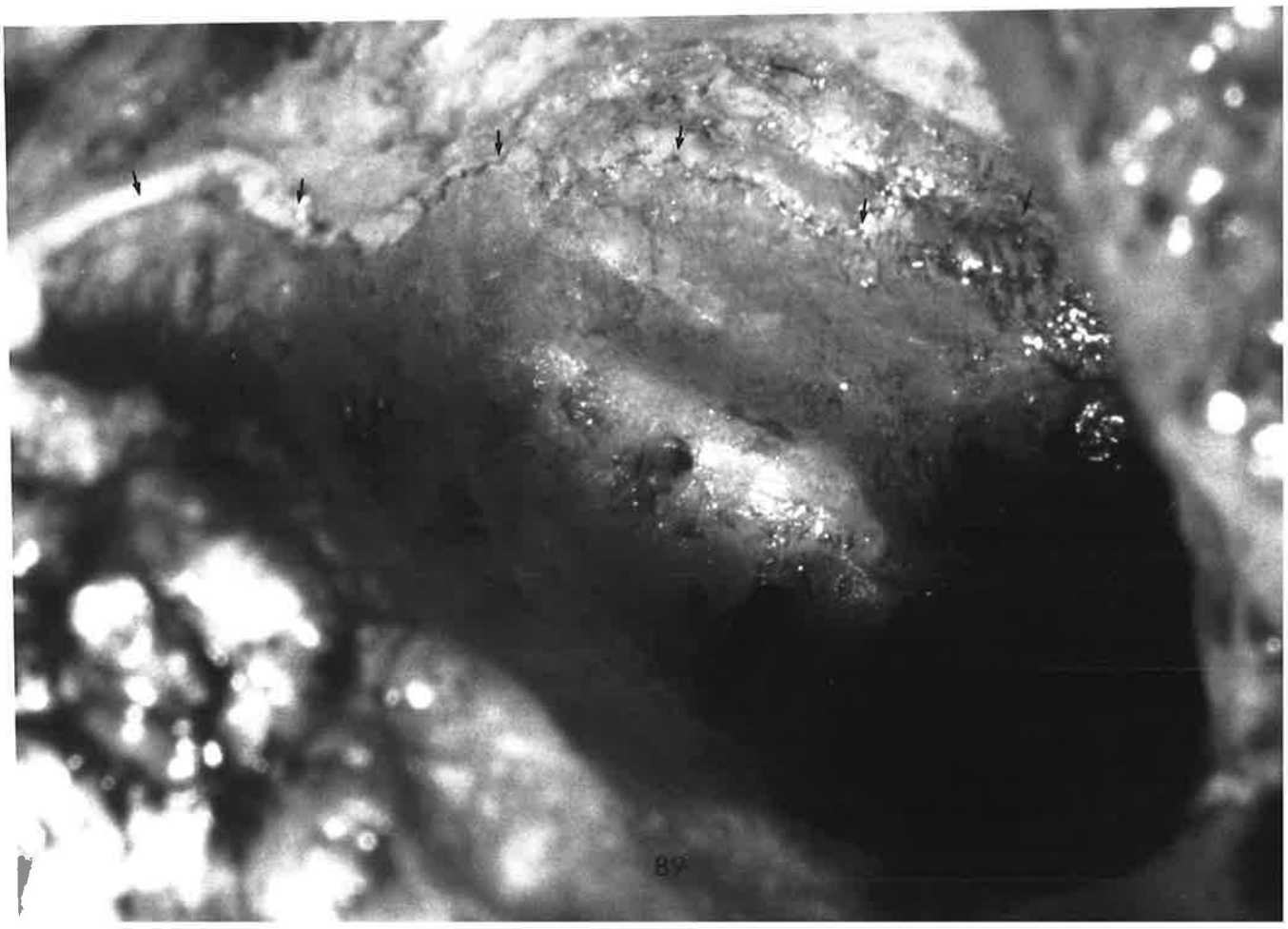
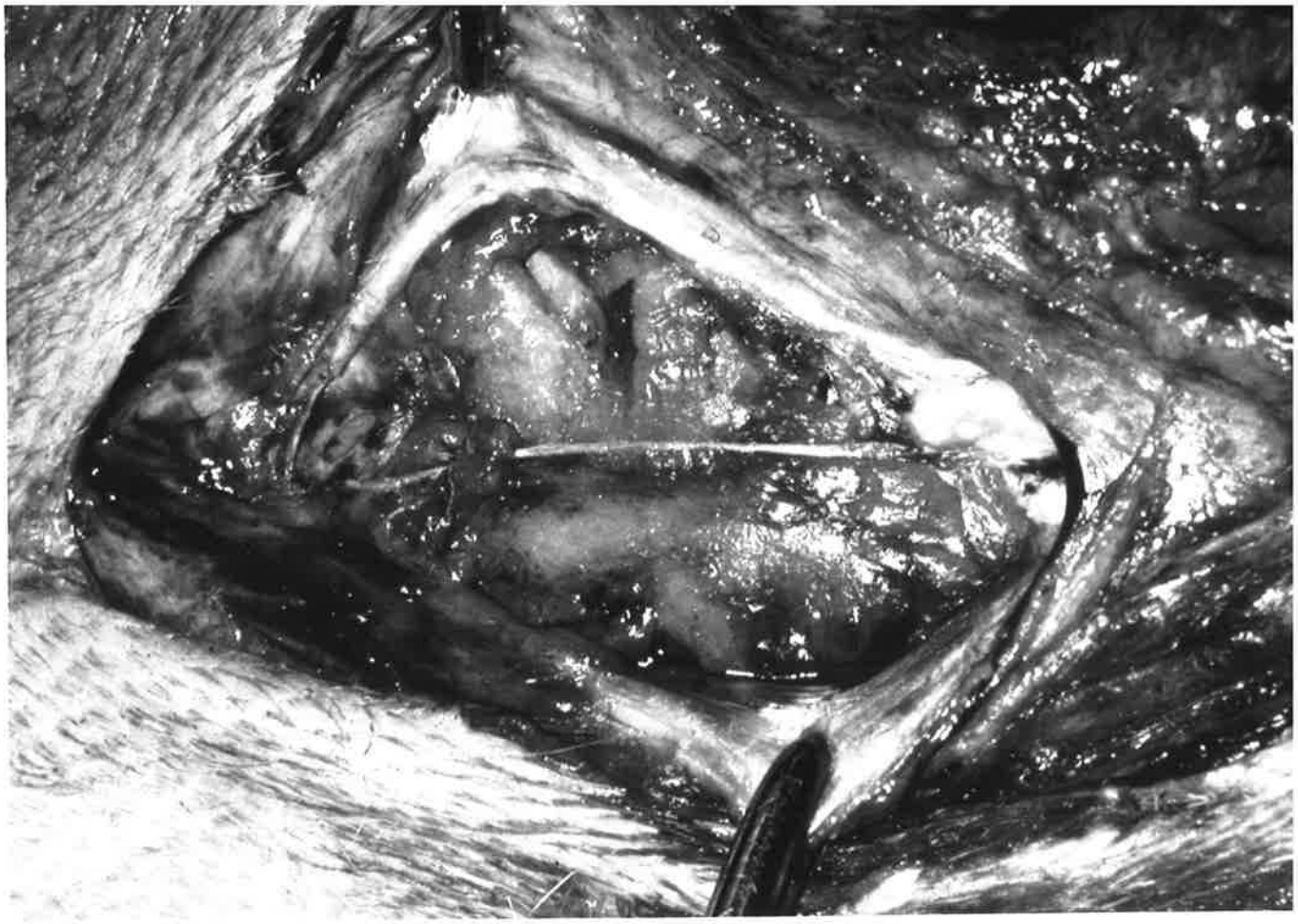


Figure 5.1.11: Arthrotomy of the ipsilateral knee joint shows a large infrapatella fat pad.

Figure 5.1.12: Incision of the fat pad and harvest of the autogenous fat to be placed in the defect.



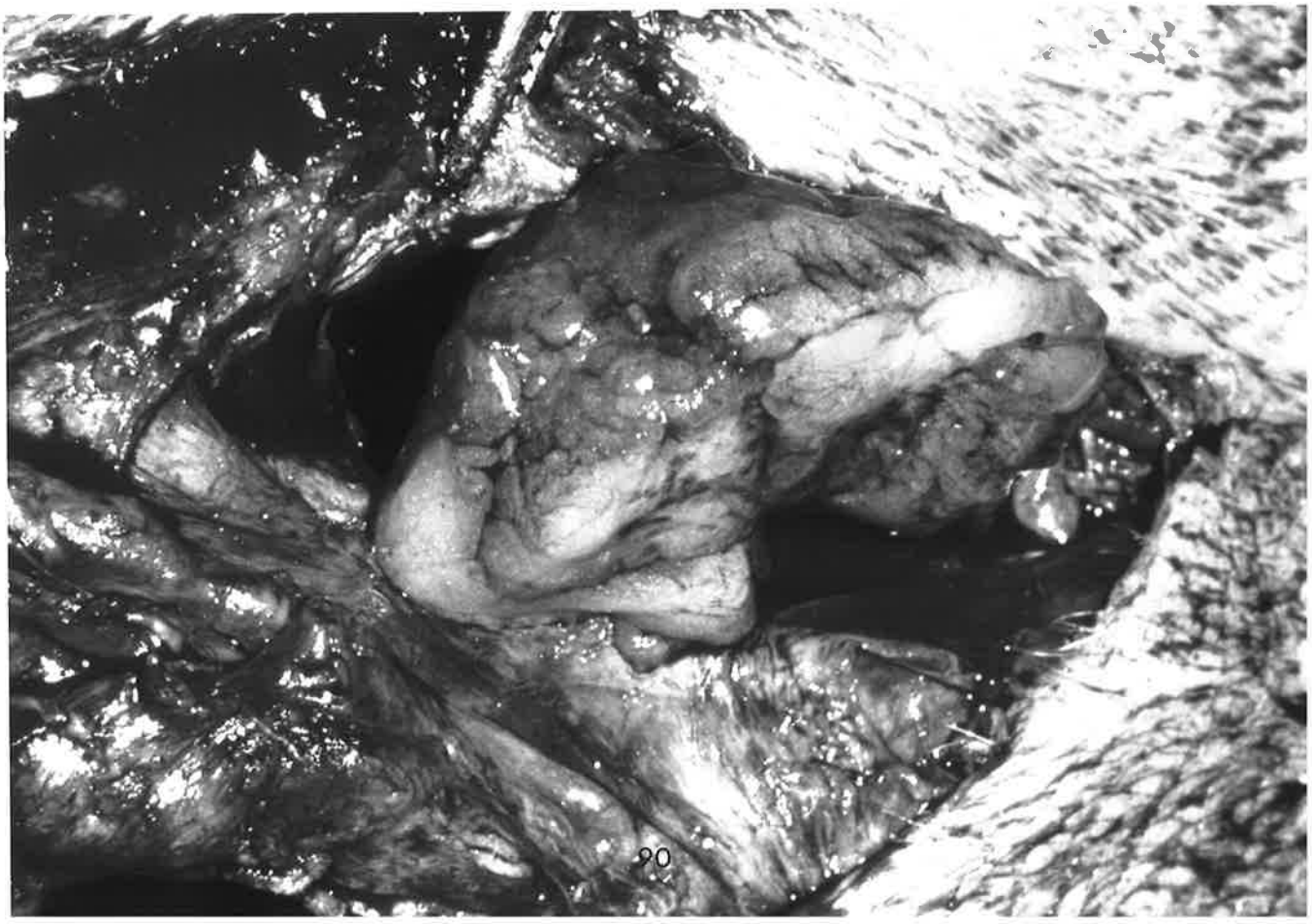
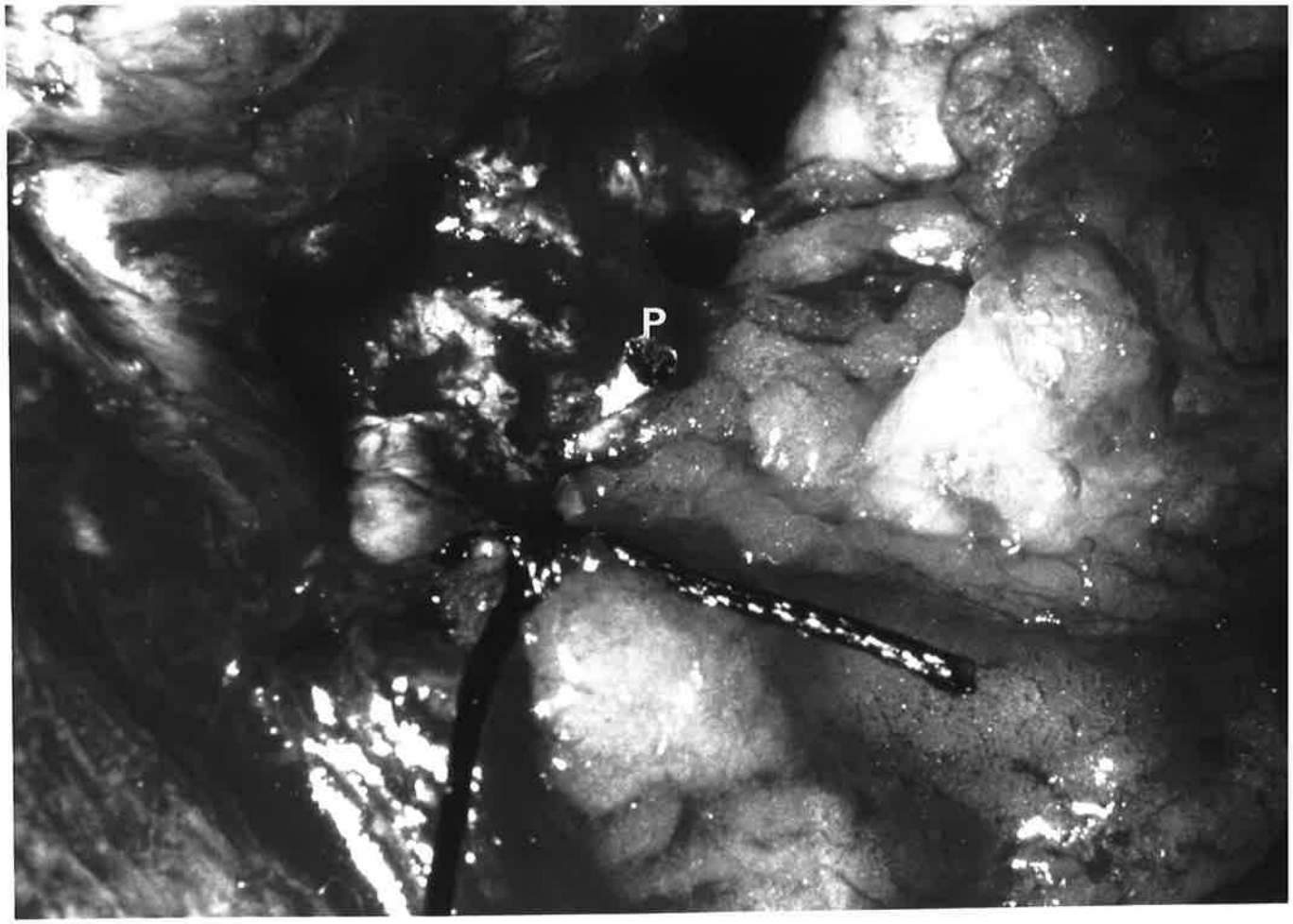


Figure 5.1.13: Suture of the fat to the epiphysis or epiphyseal marker pin to prevent loss of position of the fat once it was placed in the defect.

Figure 5.1.14: Excision of the periosteum to prevent peripheral bone bridge reformation and failure of the procedure.


P

was sutured into position with O dexon to the epiphysis or epiphyseal plate marker pin to prevent migration.

At the reversal procedure, if overgrowth of the pin tips had occurred, the epiphyseal or metaphyseal marker pins were re-sited (for example Animal 11).

After excision of the periosteum at the level of the defect the wounds were closed in layers. The previous post-operative regime was undertaken including prophylactic use of antibiotics. Weight-bearing was allowed as soon as tolerated by the animal.

Under another anaesthetic one week after the reversal procedure axial tomography (C.T.) of the tibia was performed to determine the extent of the growth plate removed. The proximal epiphyseal pin was taken as the physeal marker, and the C.T. cut was taken at 5 mm. abutting slices distal to the pin using the technique previously described.

Animals were autopsied at three or six months to examine the difference in time response to the autologous fat implants.

5.3.2 Radiological Quantification of Results:

In all x-rays the pin distance and pin angle were measured. The bone length and bone angle were also measured. The deformity index was calculated for all groups of animals at three months from the provocative procedure, and then at three months and six months after the reversal operation.

5.3.3 C.T. Quantification of Results:

At autopsy a repeat C.T. of the tibia was undertaken and results were compared to the initial slice. Using computerized digital planimetry the percentage of defect area to the plate area was estimated three times, and the median taken (Fig. 5.2).

Statistical analysis using the unpaired Student's *t* test was used to assess difference in C.T. areas.

5.4 HISTOLOGICAL QUANTIFICATION OF RESULTS:

The histological preparation was as previously described. The whole bone sections were reviewed and quantification of the histology determined.

a. Assessment of the response of the host tissue to the implant:

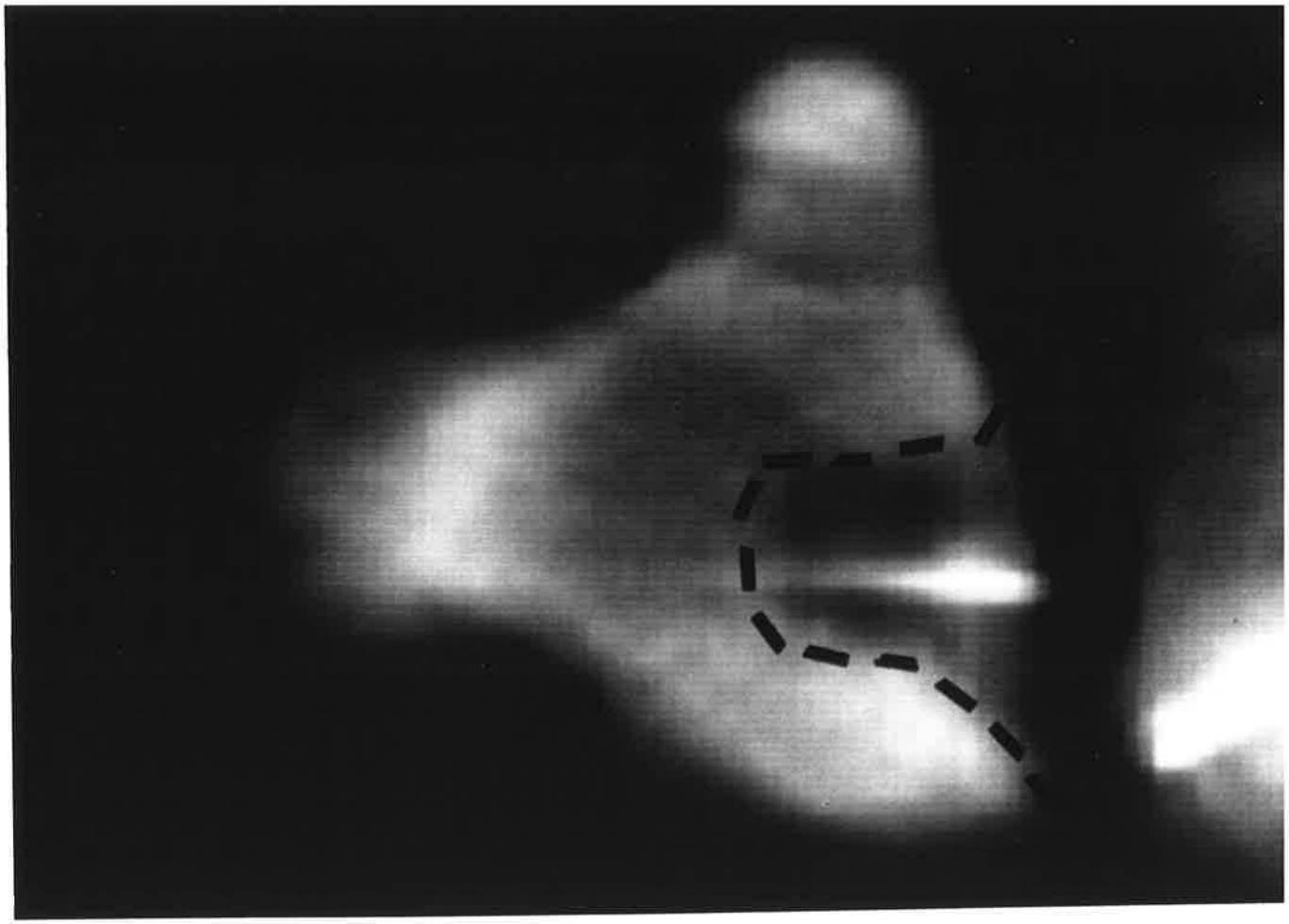
Firstly, the degree of fibrosis around the fat implant was determined. It was graded as zero if absent, one plus (+) if present, and two pluses (++) if there was an organized capsule (Fig. 5.3).

Secondly, the remodelling activity of the host cancellous bone of the epiphysis and metaphysis was estimated by the degrees of osteoblastic activity. There were 3 grades of remodelling: zero showed no osteoblastic activity; one plus (+) indicated active bone formation without increase of trabecular width; two pluses (++) indicated active bone formation with increased trabecular width (Fig. 5.4).

Figure 5.2: C.T. QUANTIFICATION OF PHYSEAL AREA.

Figure 5.2.1: The C.T. slice that just included the proximal pin was taken as the representative slice for calculation of areas. On the hard copy the defect area to be calculated was outlined (broken line).

Figure 5.2.2: Using a floppy disc a digital planimeter was used to calculate the relative surface areas. The percentage of defect to total area was calculated 3 times and the mean obtained.



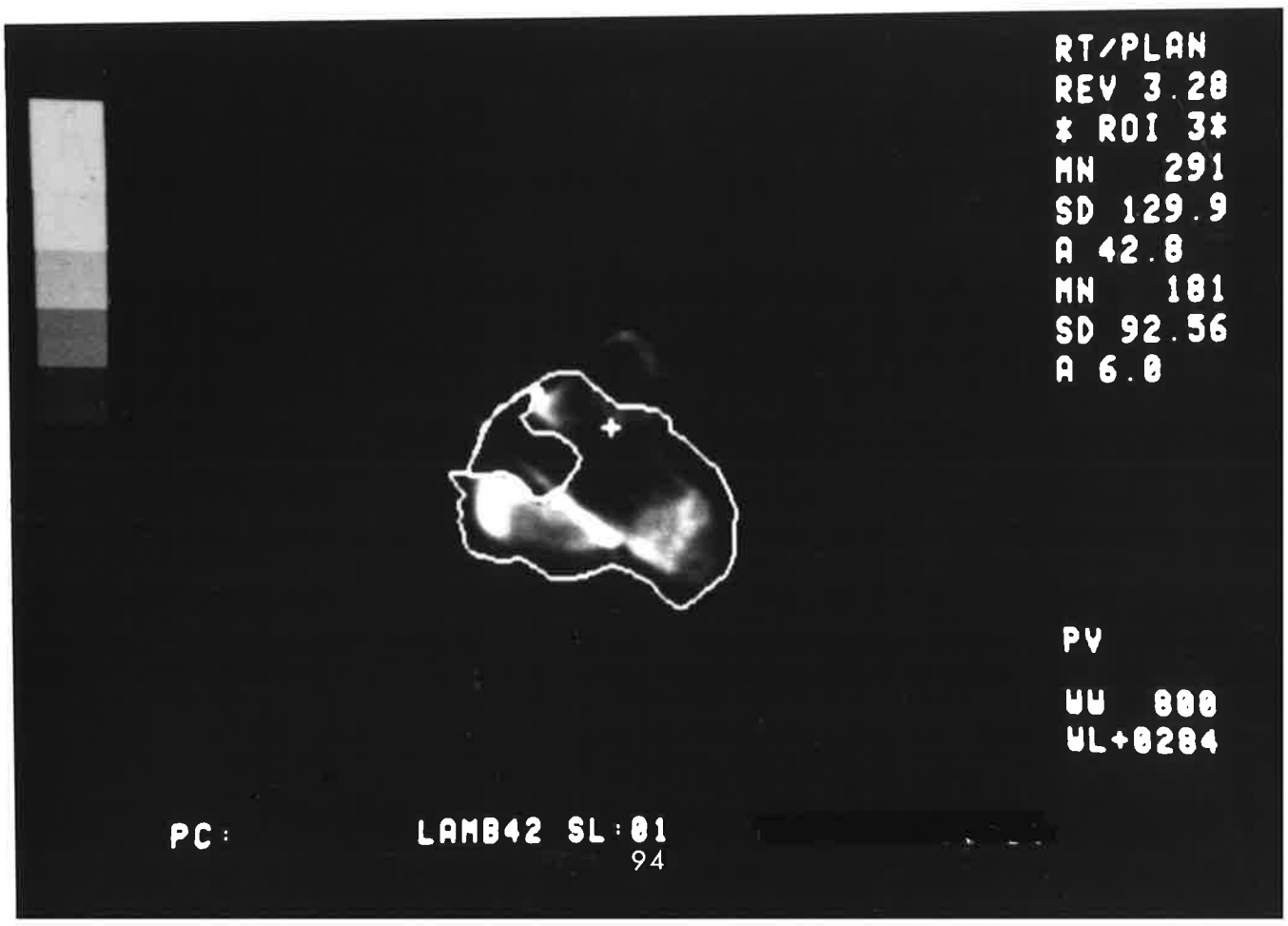

RT/PLAN
REV 3.28
* ROI 3*
MN 291
SD 129.9
A 42.8
MN 181
SD 92.56
A 6.0
PV
WW 800
WL+0284
PC:
LAMB42 SL:01

Figure 5.3: HISTOLOGICAL ASSESSMENT OF HOST TISSUE RESPONSE TO THE IMPLANT - CAPSULE FORMATION.

Figure 5.3.1: The degree of fibrosis around the viable fat can be seen in this whole tibial section of Animal 20. (x 3)

The higher field (x 325) grade was 0. There is a small rim of fibrous tissue between the fat and the host.

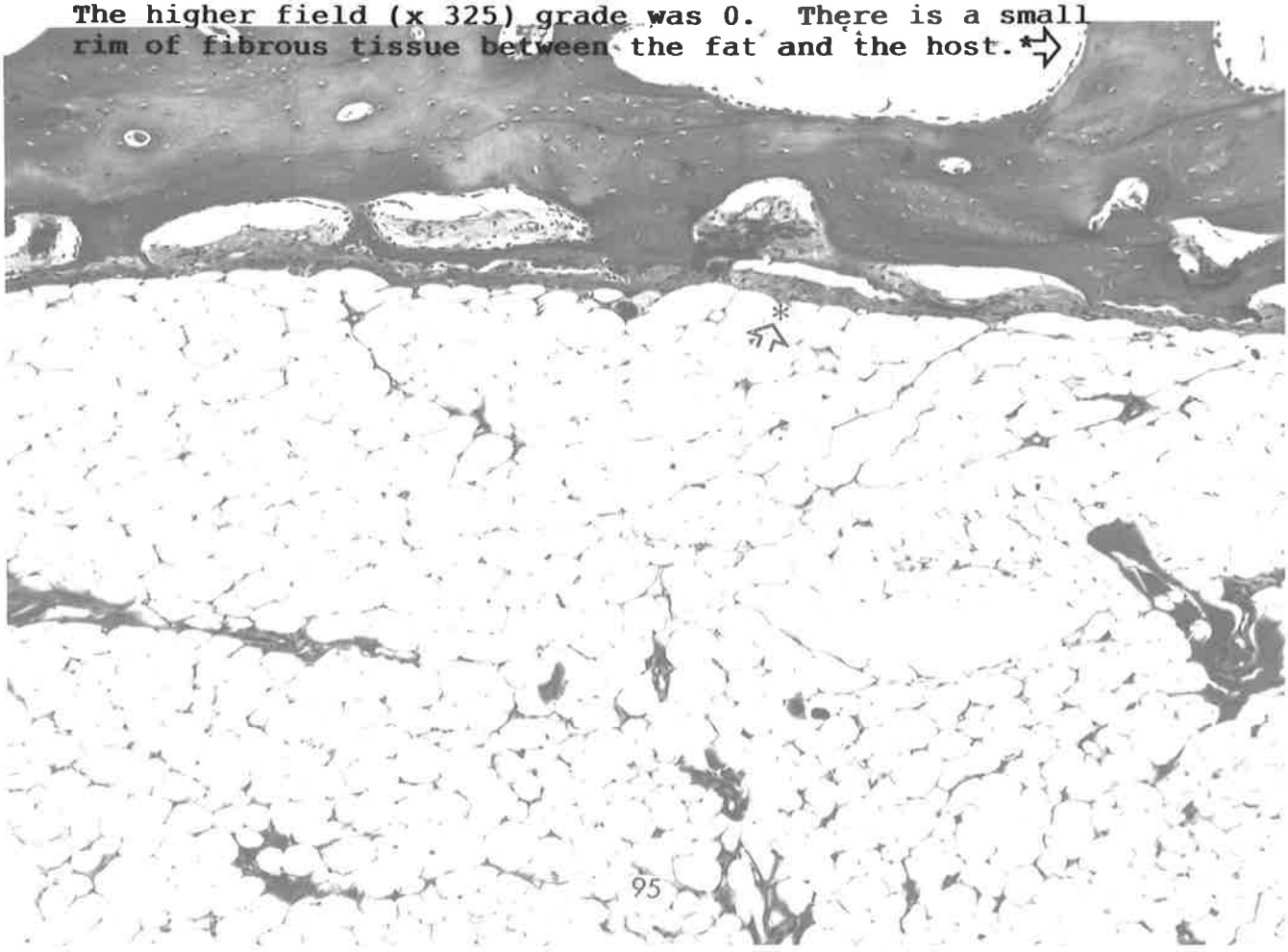


Figure 5.3.2: One plus (+) grade of fibrosis with collagen fibres and fibroblasts as seen in Animal 17. (x 250) ⇨

Two plus (++) grade of fibrosis with formation of an organized capsule in Animal 26. (x 325) ⇨

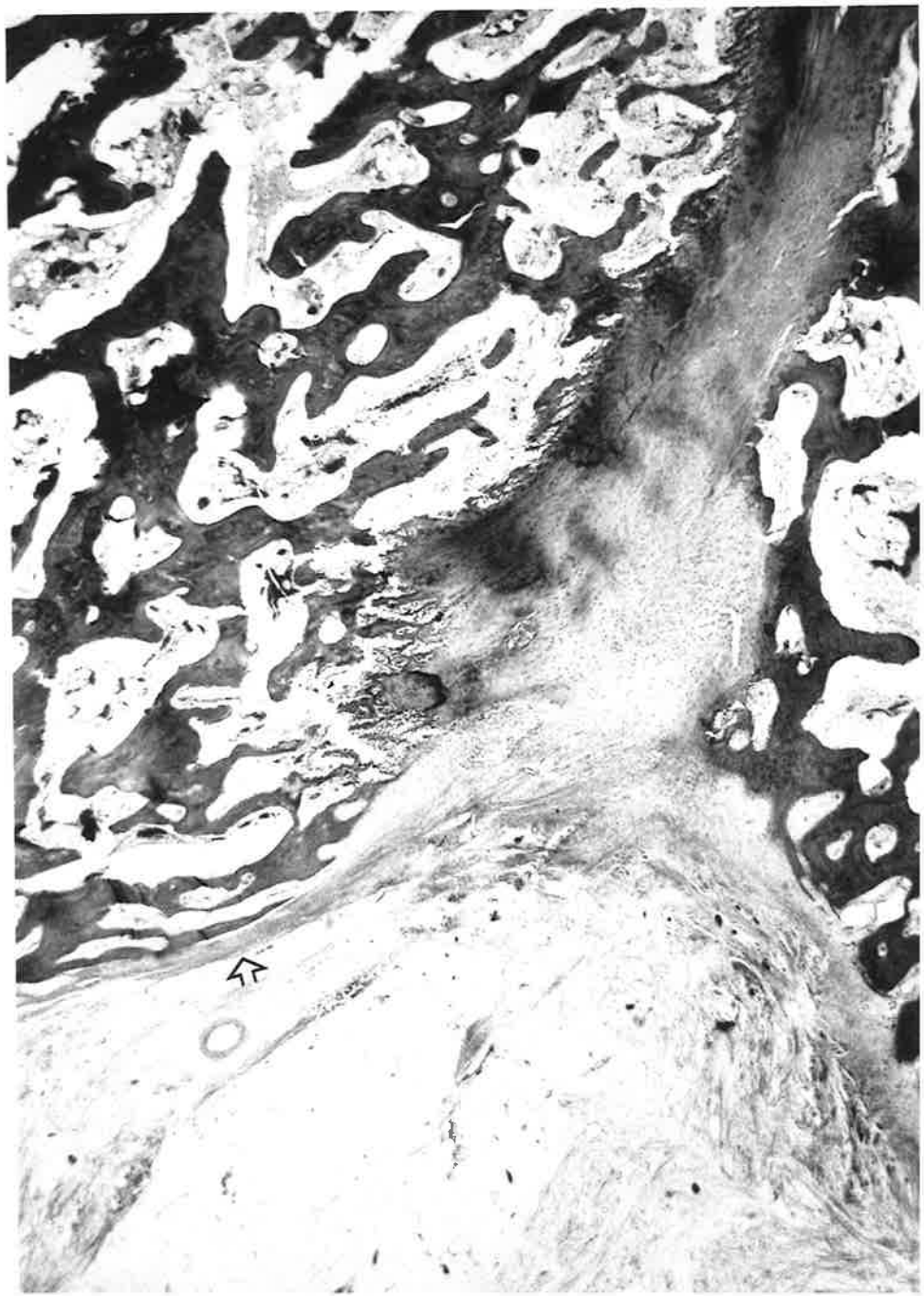
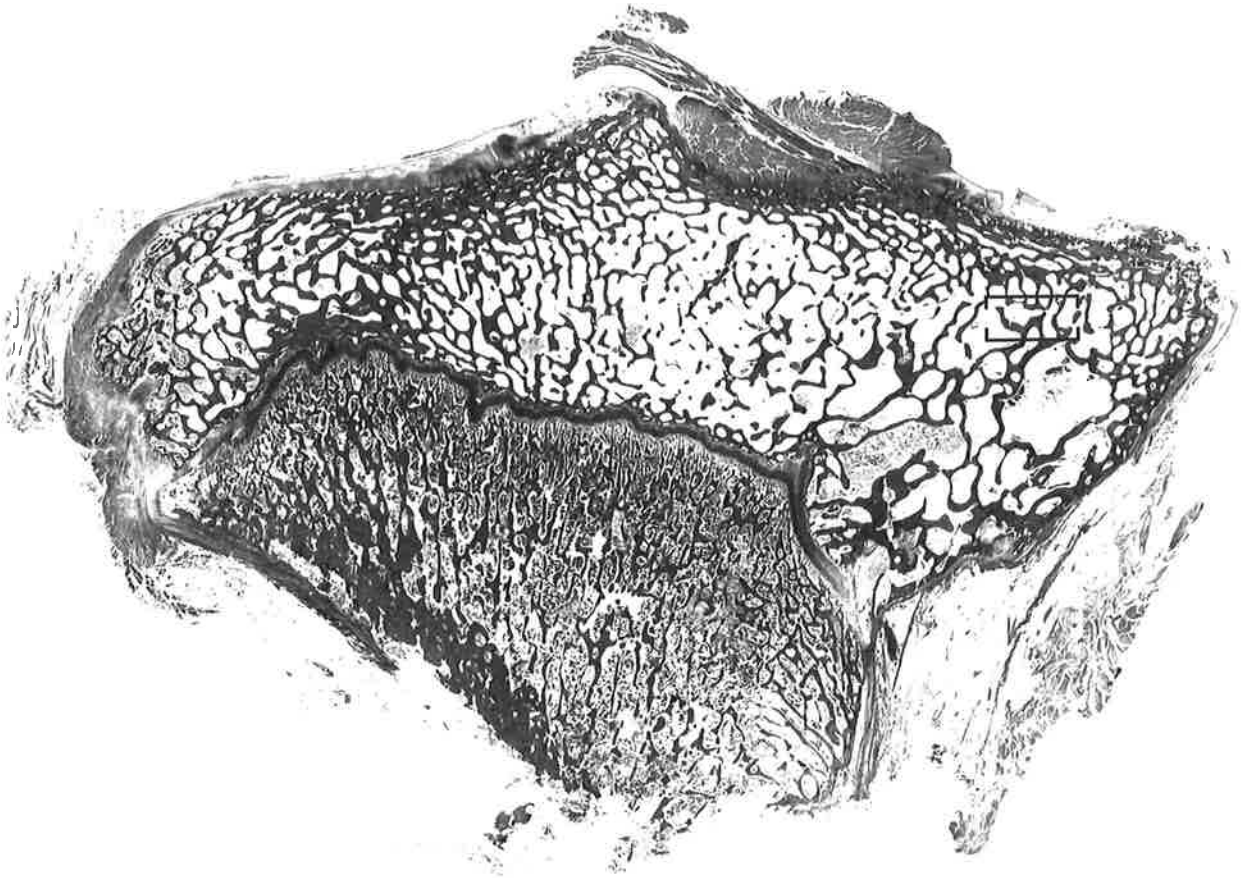


Figure 5.4: HISTOLOGICAL ASSESSMENT OF HOST TISSUE REACTION TO THE IMPLANT - EPIPHYSEAL BONE RESPONSE.

Figure 5.4.1: The degree of epiphyseal new bone formation can be seen in this whole tibial section of Animal 18. (x 3)

The higher power field grade was 0. There is no trabecular bone thickening and little osteoblastic activity. (x 325)



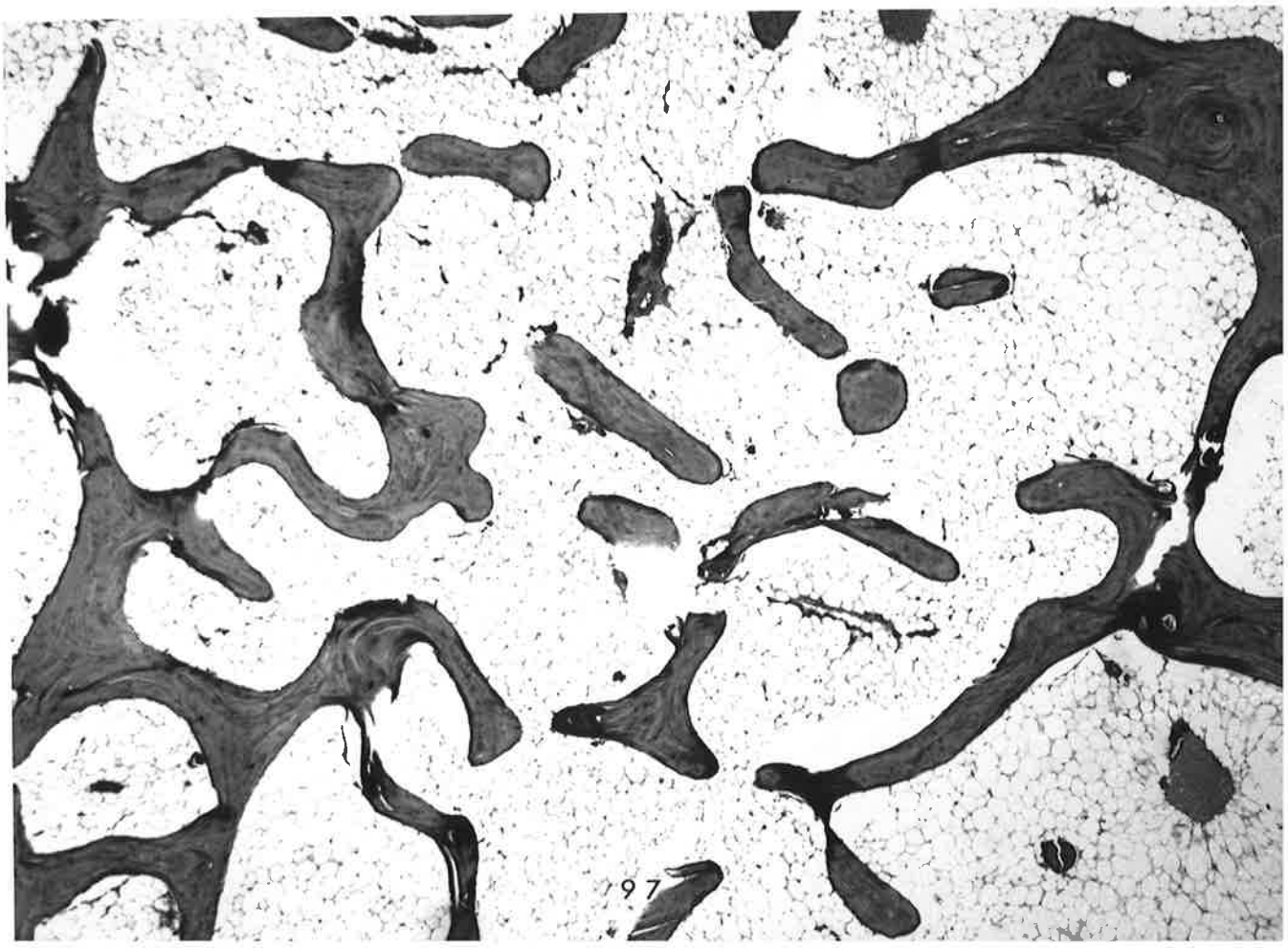
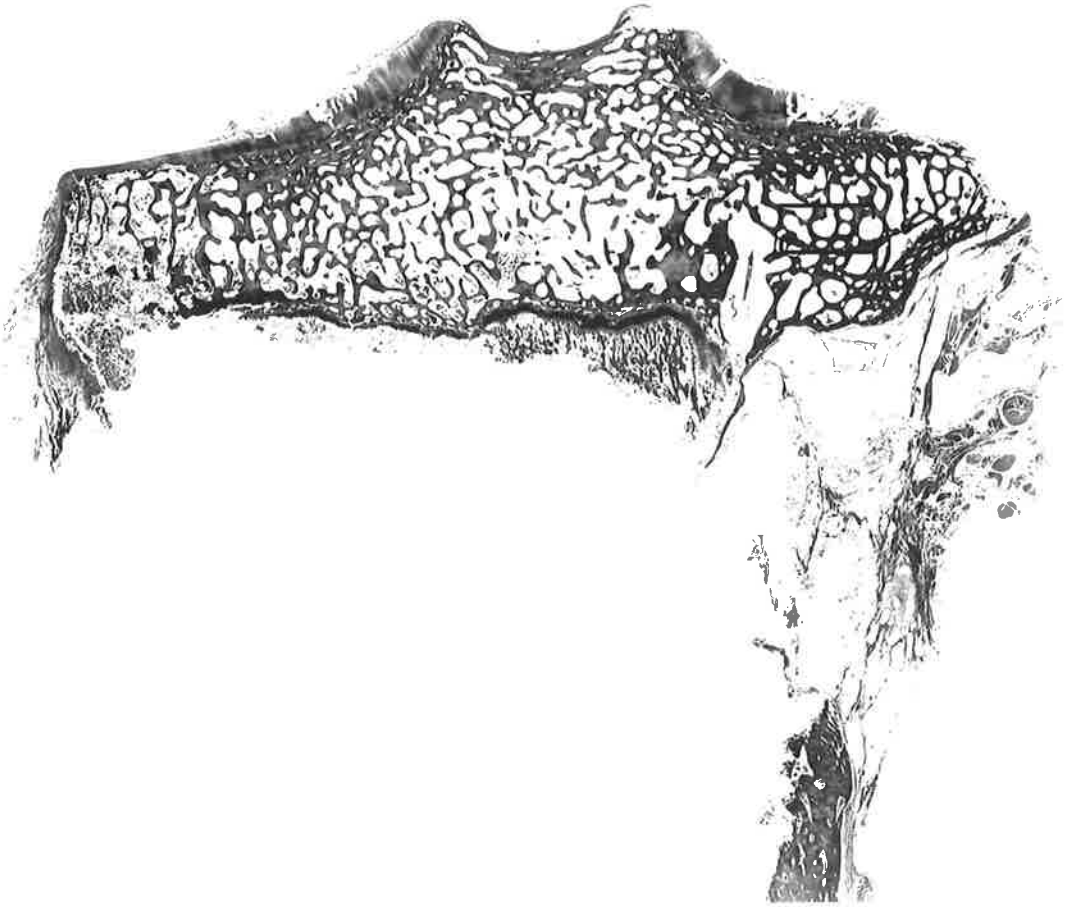


Figure 5.4.2: (+) Epiphyseal bone formation as seen in the whole tibial section of Animal 20. (x 3)

The higher power field (x 325): Note the increased width of trabeculae and osteoblastic activity.



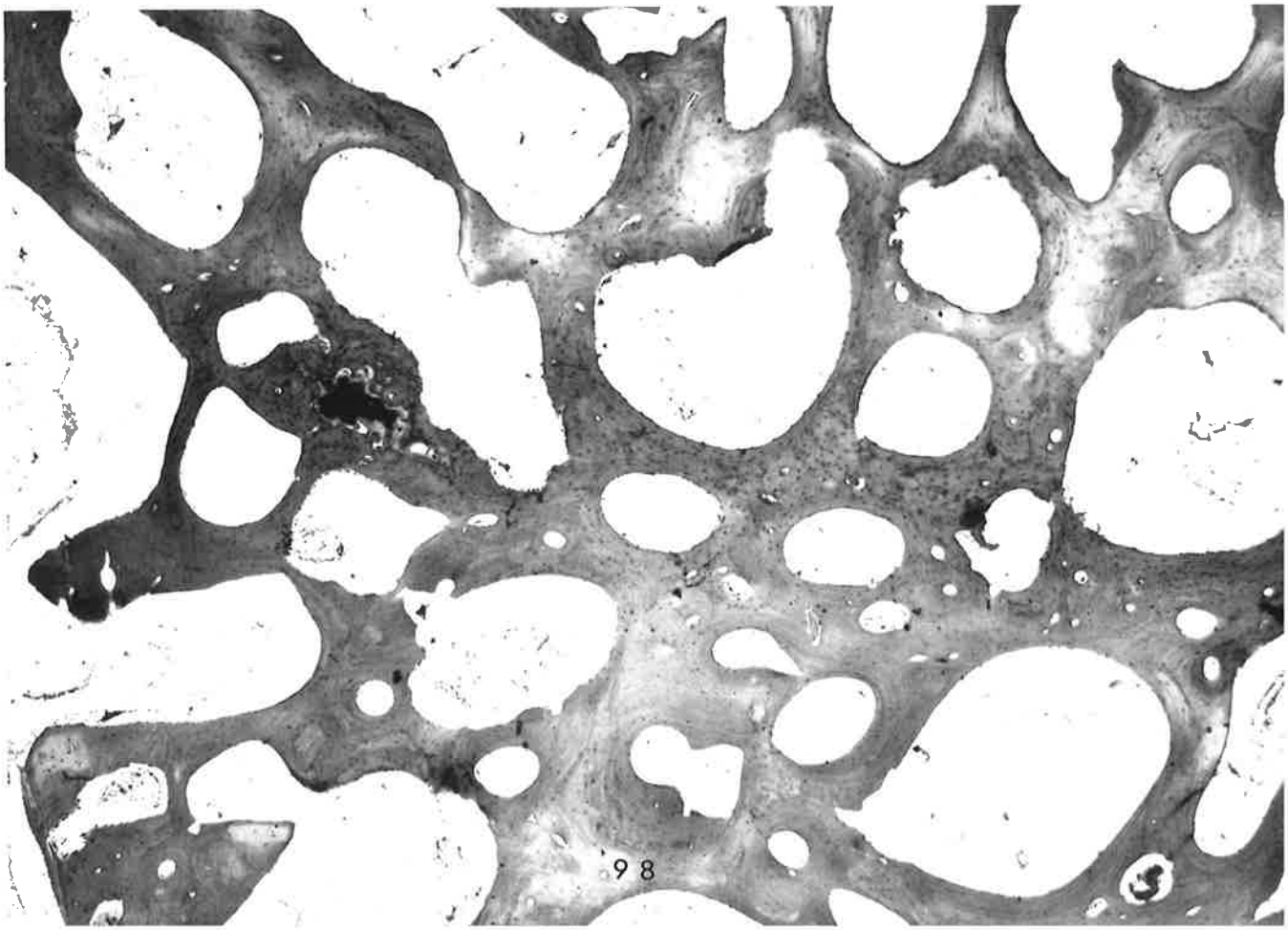
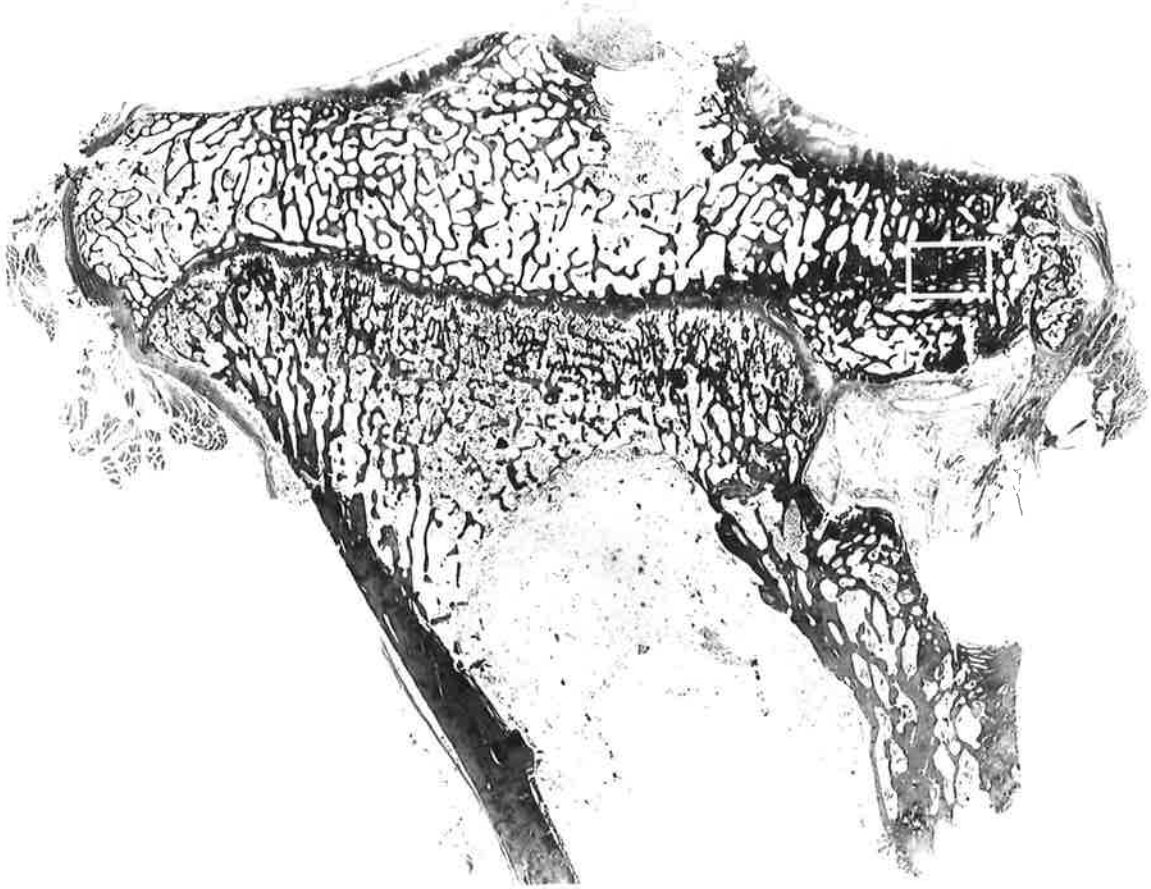
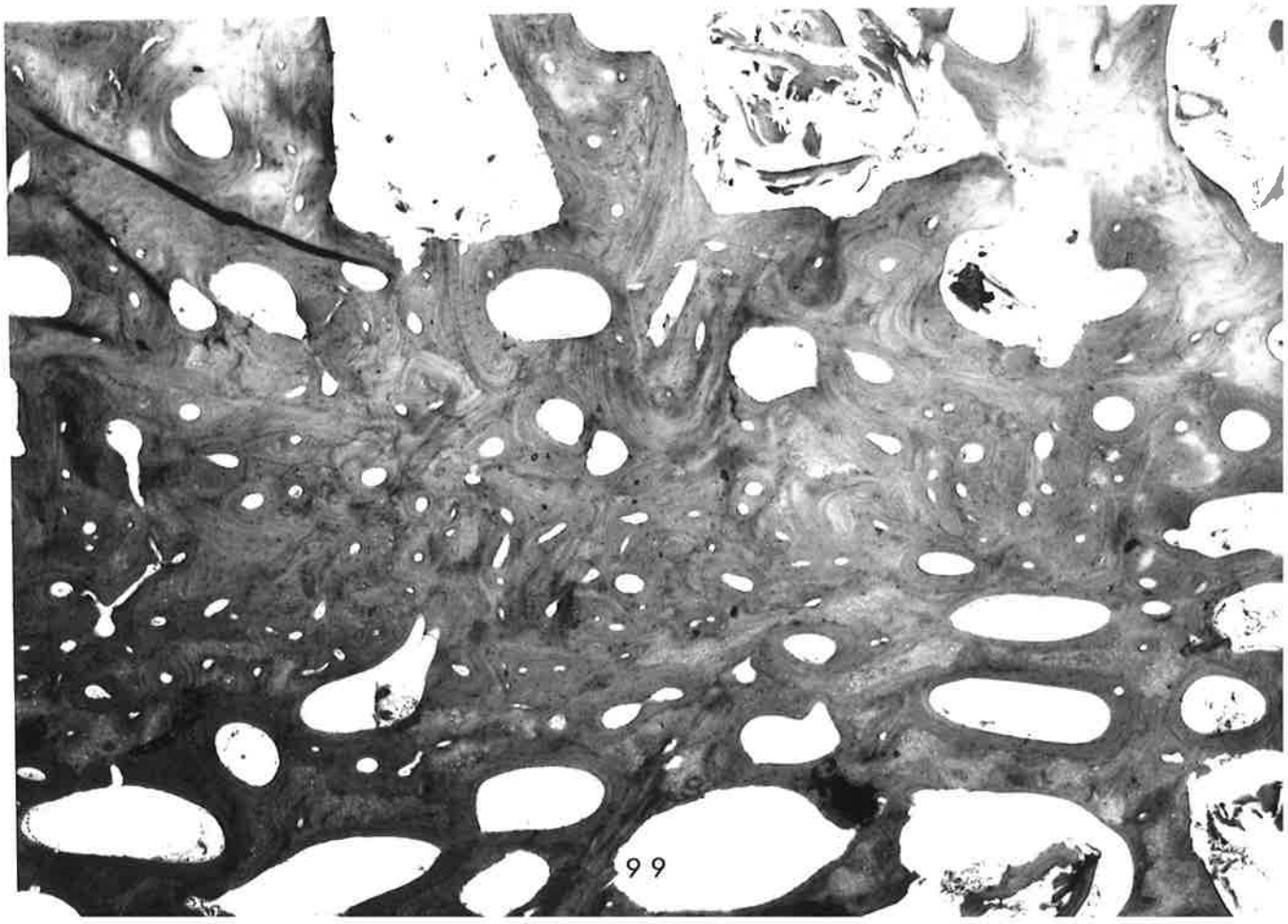


Figure 5.4.3: (++) Epiphyseal bone formation as seen in the whole tibial section of Animal 17. (x 3)

The higher powered field (x 325) Note the more than double width of trabeculae and loss of inter-trabecula space.





Thirdly, the cortical bone thickness of the metaphyseal bone adjacent to the defect was graded as zero (same), (+) intermediate, (++) double thickness of normal cortical bone (Fig. 5.5).

b. The fate of the fat:

Firstly, fat was assessed for preservation of site of the implant: whether it remained physeal (P), or metaphyseal (M), or epiphyseal (E) in situ. Secondly, the viability of the fat graft and any change in size was graded: (i) (N) normal, (ii) H hypertrophy, (iii) P/N partial necrosis, (iv) C/N complete necrosis. Thirdly, the extent of interstitial fibrosis was rated as absent (0), present (+), marked (++) (Fig. 5.6).

c. The recurrence of bone bridge fusion:

Any bone bridge reformation was identified and the site, either peripheral or central determined. The bone bridge was then classified as showing cancellous woven bone or cortical bone (Fig. 5.7).

d. The response of the host physis:

The morphological features of the physeal contour were assessed.

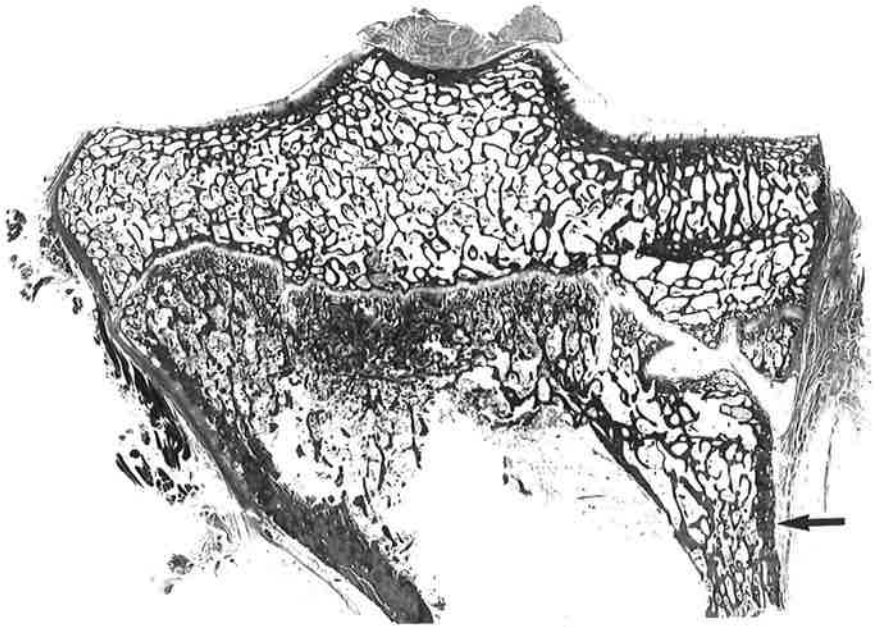
Firstly, the thickness of the physis immediately adjacent to the fat graft showed a medial spur of physeal tissue. This was graded as either (i) absent (0), (ii) present (+), or (iii) markedly elongated (++) (Fig. 5.8).

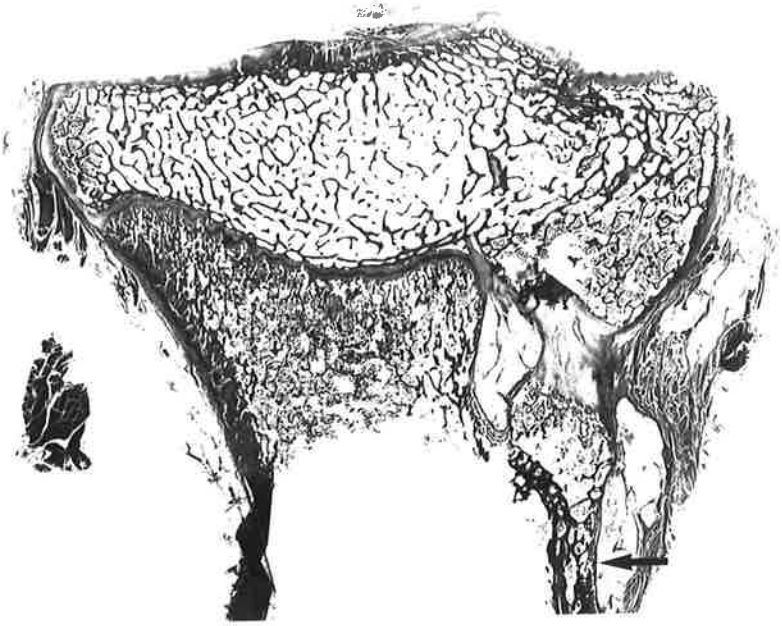
Figure 5.5: HISTOLOGICAL ASSESSMENT OF HOST TISSUE REACTION TO THE IMPLANT - CORTICAL BONE REMODELLING.

Figure 5.5.1: (0) grade. The cortical bone is the same width. There may be an increase in the cancellous bone of the metaphysis. (x 2.5)

Figure 5.5.2: (+) grade. As seen in Animal 26 where the cortical bone is increased in width. (x 2)

Figure 5.5.3: (++) grade. As seen in Animal 29 the cortical bone has increased in size at least twice the width of the other cortex. (x 2.5)





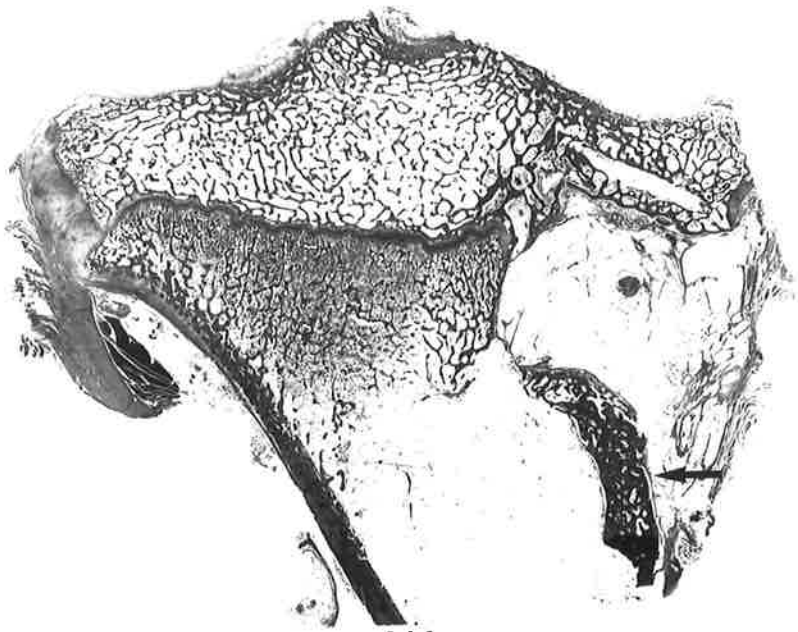
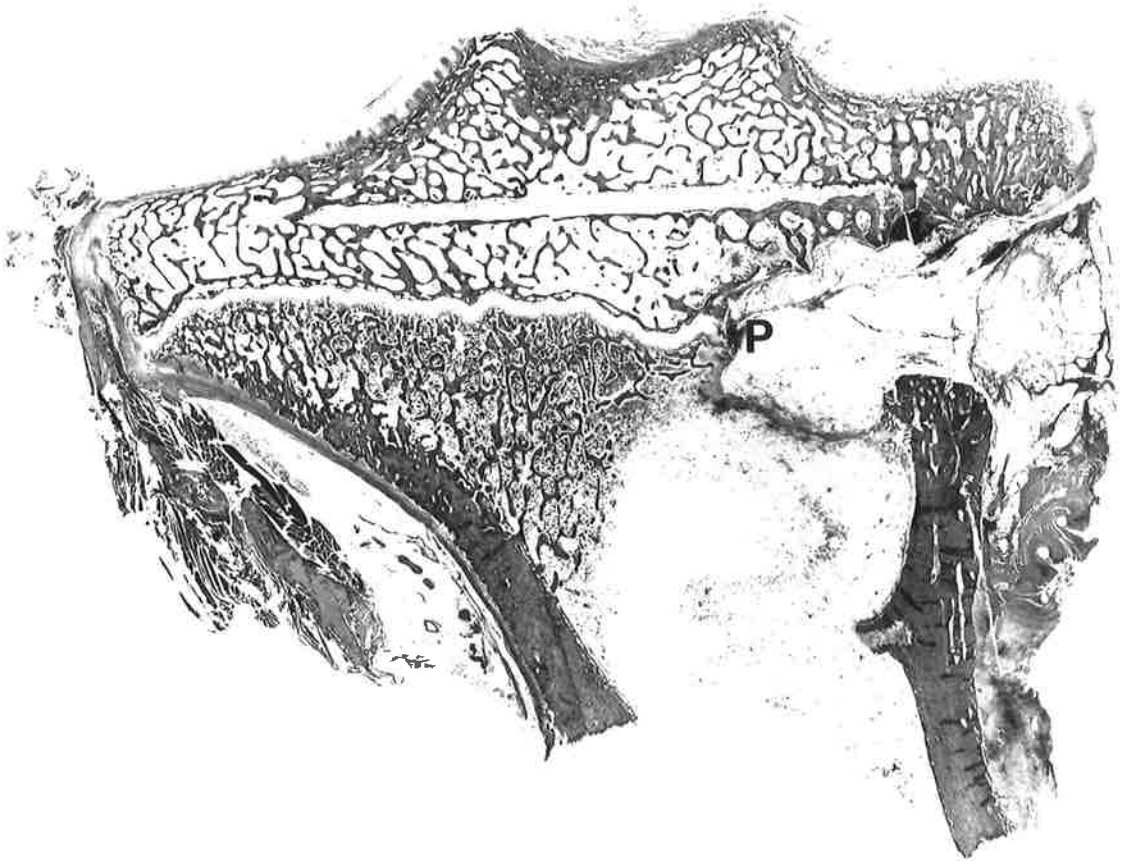


Figure 5.6: HISTOLOGICAL ASSESSMENT OF THE FAT AUTOGRAFT.

Figure 5.6.1: Normal fat graft in situ at the physeal level (P) without apparent increase in size. (x 3)

Figure 5.6.2: Animal 20 demonstrates elongation of the defect at 6 months from the initial reversal procedure. It was interpreted as evidence of hypertrophy of the graft with graft remaining in contact with the normal epiphyseal bone. (x 3)


P

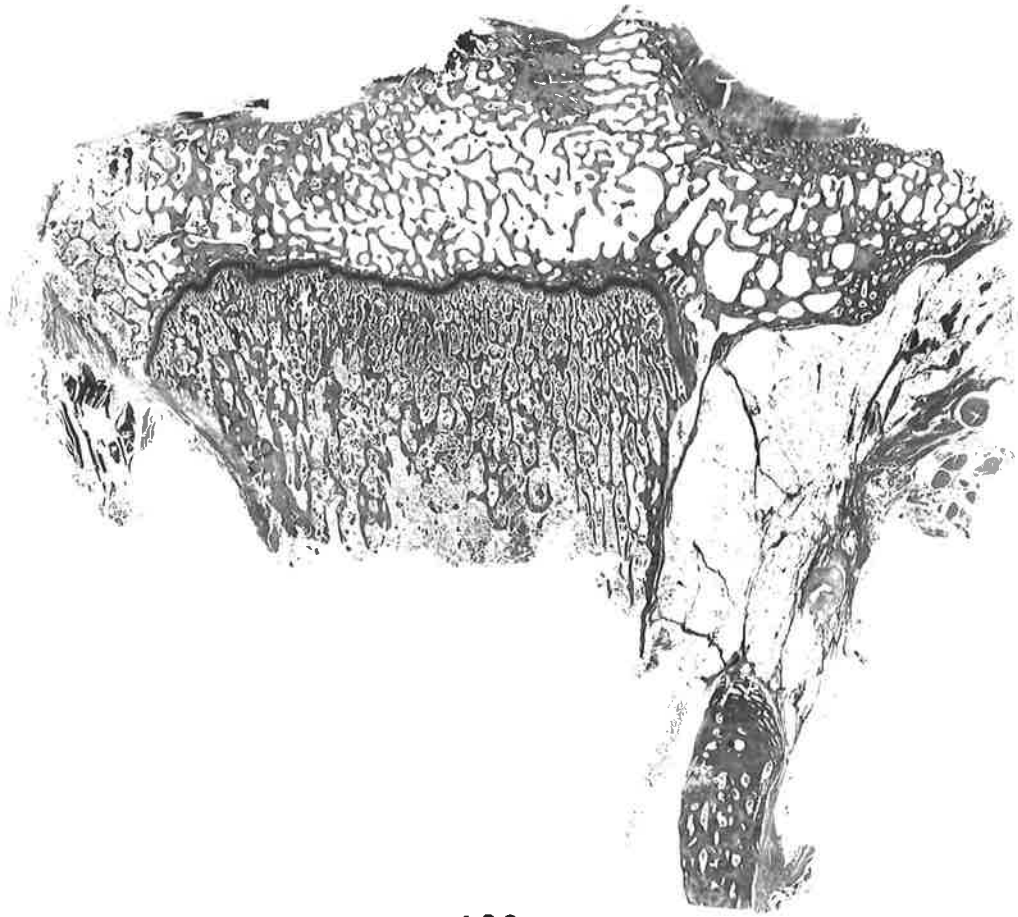
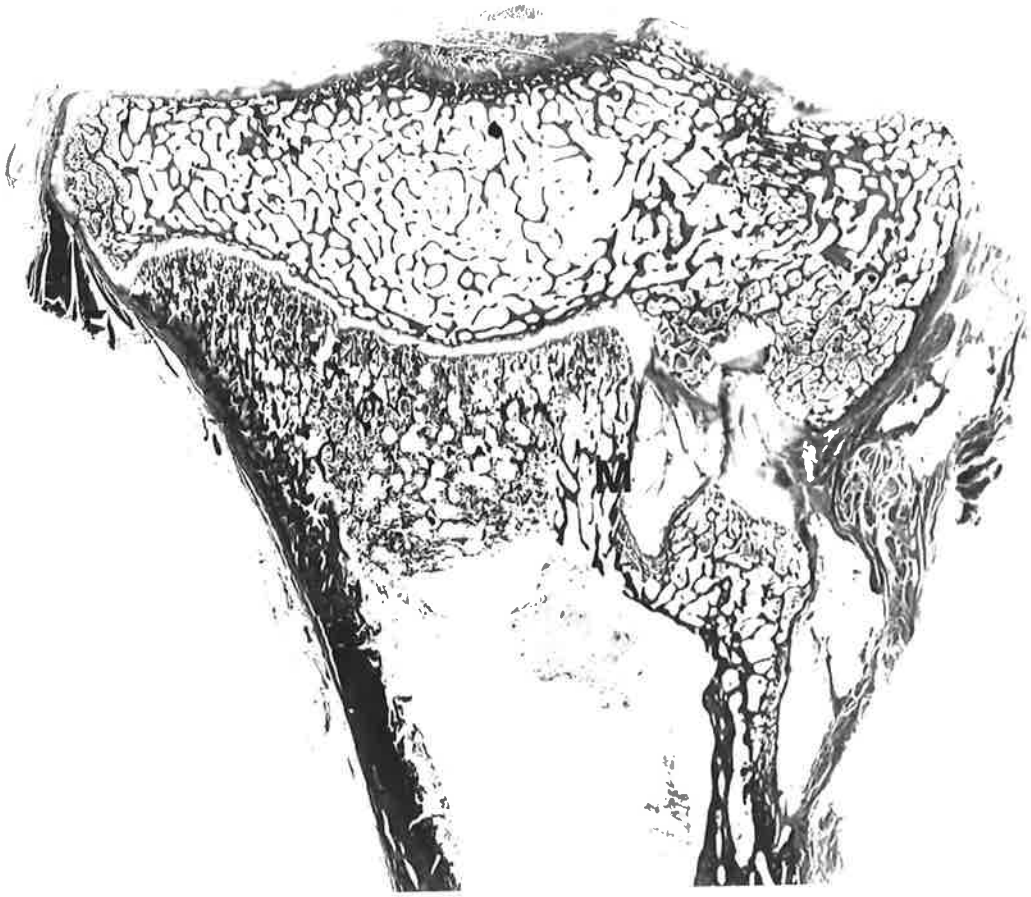


Figure 5.6.3: Animal 26 demonstrates partial necrosis of the fat in the defect at 3 months with interstitial fibrosis (+) and capsular formation.

The graft is more metaphyseal (M) in location. (x 3)

Figure 5.6.4: Animal 31 demonstrates complete necrosis of the fat with marked insterstitial fibrosis (++).N

There are additional features in the epiphyseal and metaphyseal bone that show bone remodelling. (x 3)


M

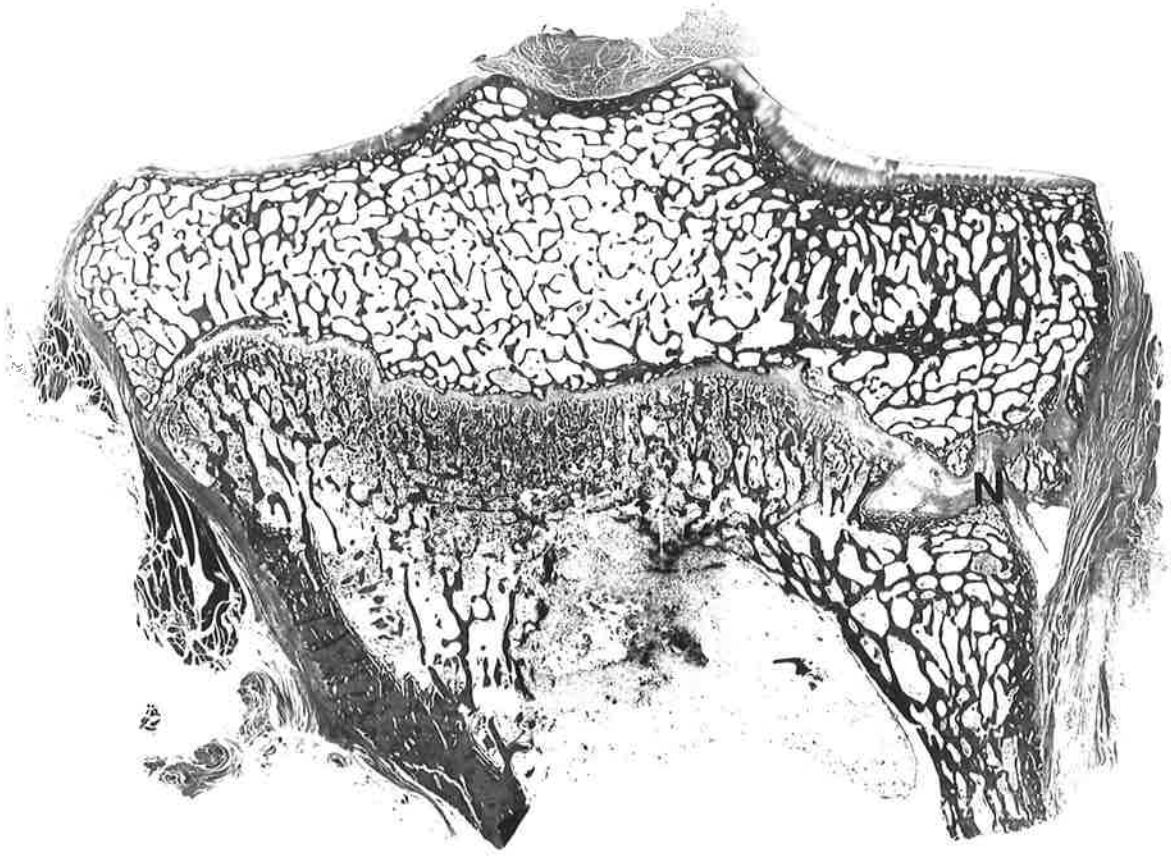
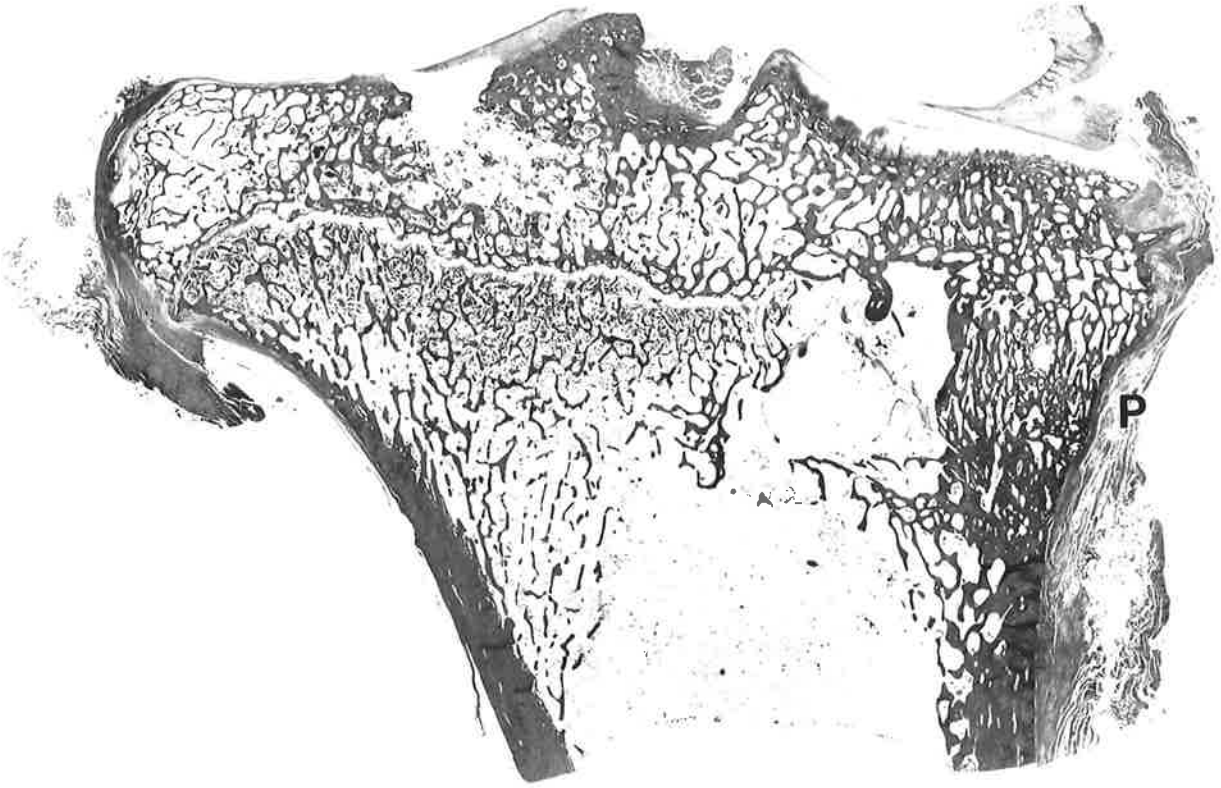

N

Figure 5.7: **HISTOLOGICAL ASSESSMENT OF BONE BRIDGE REFORMATION.**

Figure 5.7.1: Peripheral bone bar formation.P In Animal 38 the cancellous bone bar can be seen joining the epiphysis and metaphysis. A viable fat defect is present at the physeal level. (x 3)

Figure 5.7.2: Central bone bar formation.C In Animal 42, where there is a large physeal defect of greater than 1/3rd of the diameter in this plane, a complete cancellous bone bridge is demonstrated. (x 3)


P

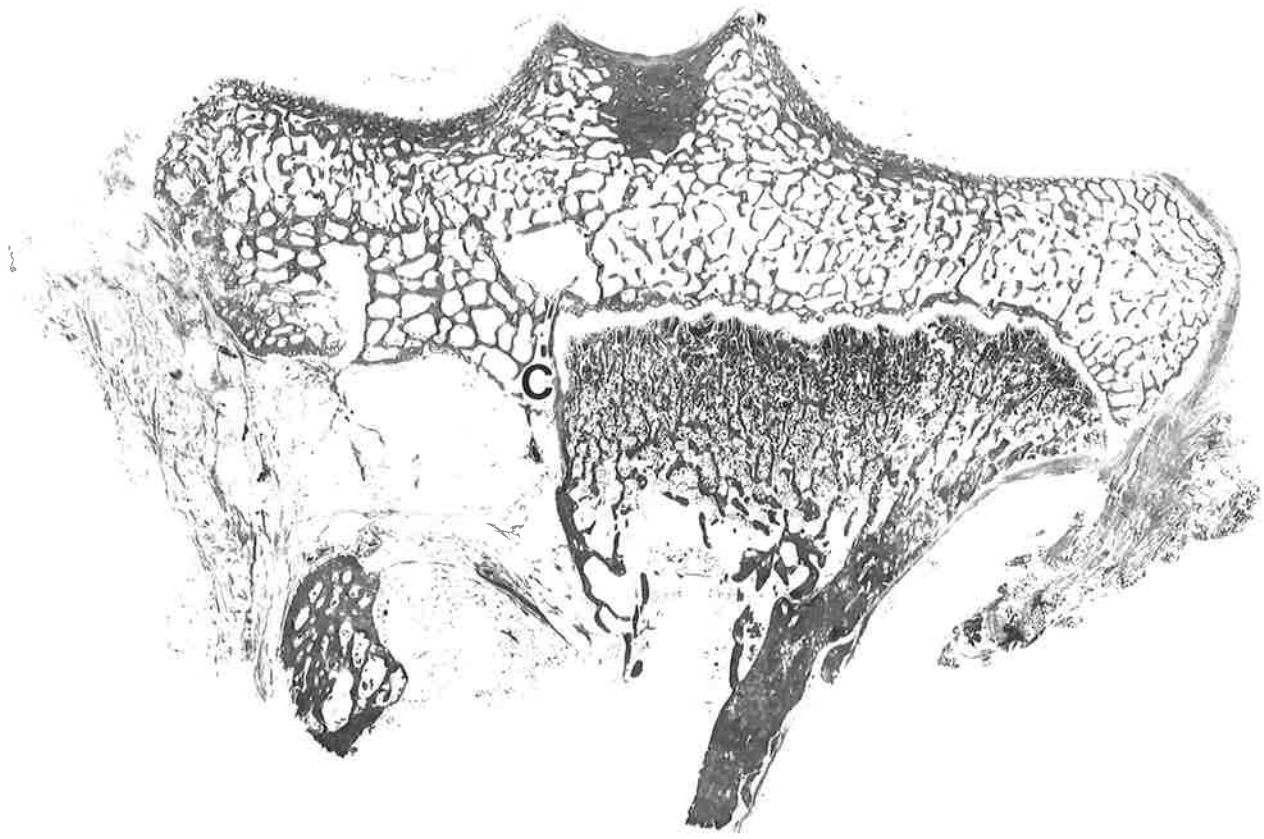
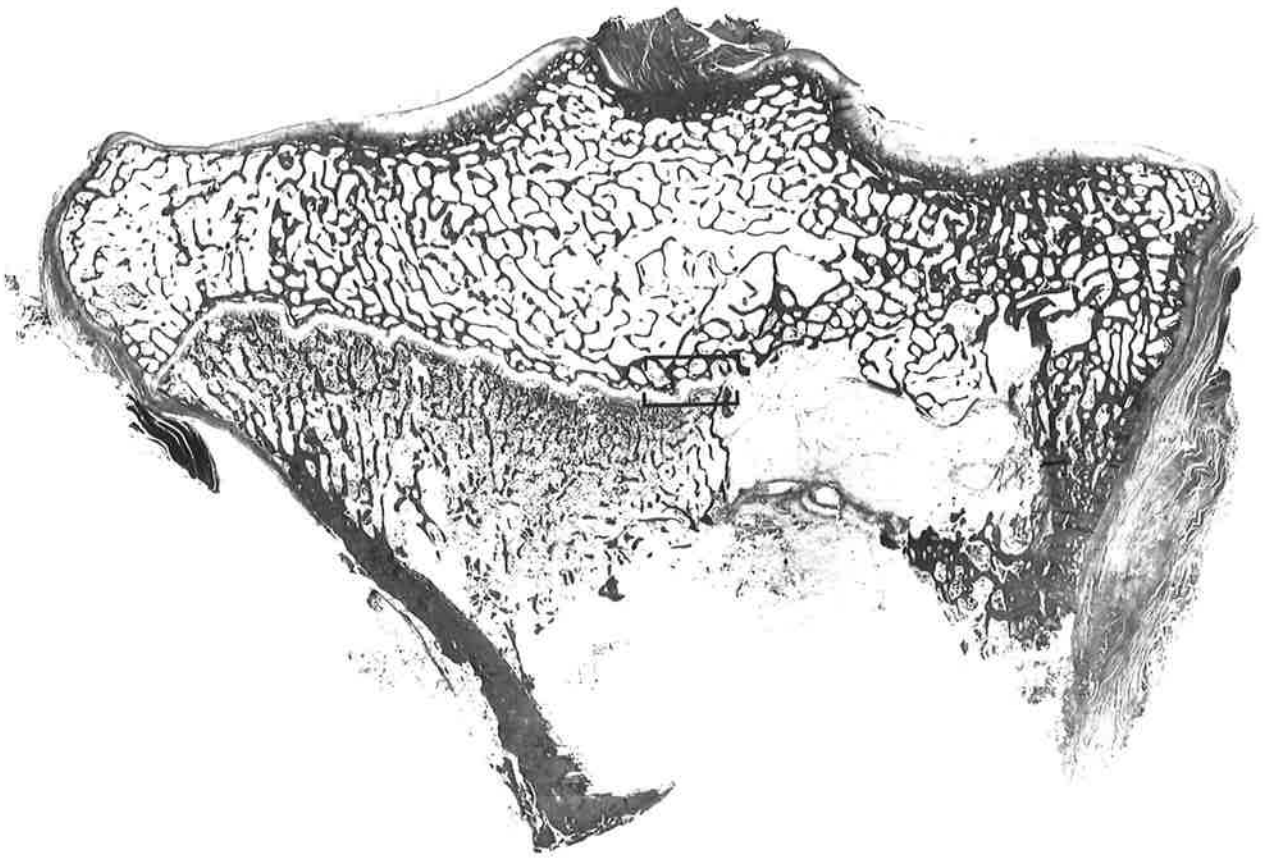

C

Figure 5.8: HISTOLOGICAL ASSESSMENT OF HOST PHYSEAL RESPONSE.

Figure 5.8.1: Animal 38 demonstrates an indolent physeal response. There is no spur of medial tissue.(x 3)

High power field illustrates the lesion and lack of physeal hypertrophy. (x 325)



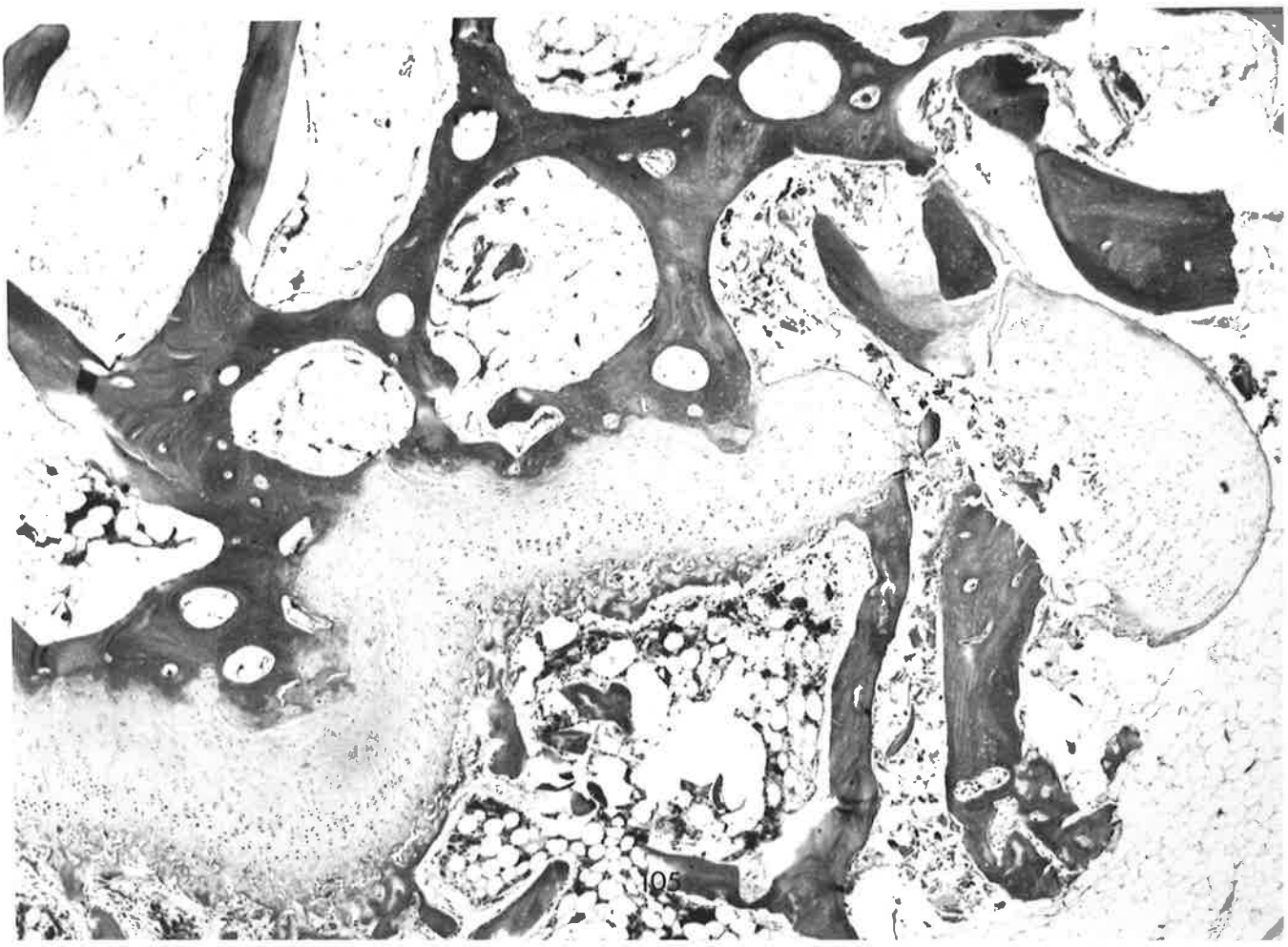
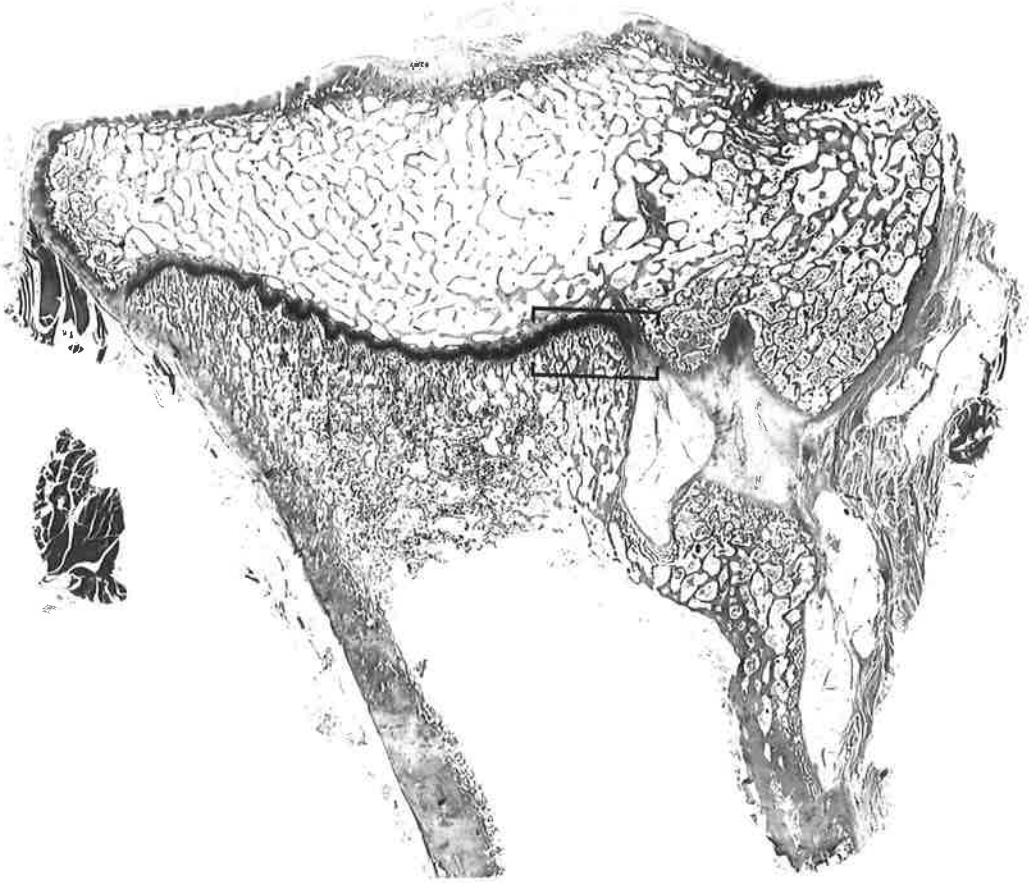


Figure 5.8.2: A complete section of the upper tibia in Animal 26 demonstrates a typical physeal response of hypertrophy of physeal tissue grade (+). (x 3)

High power of the inner field demonstrates increase in width of the physis with a medial spur of cartilage-like material extending into the metaphysis. There is loss of the normal cellular regularity of the physeal contour. (x 325)



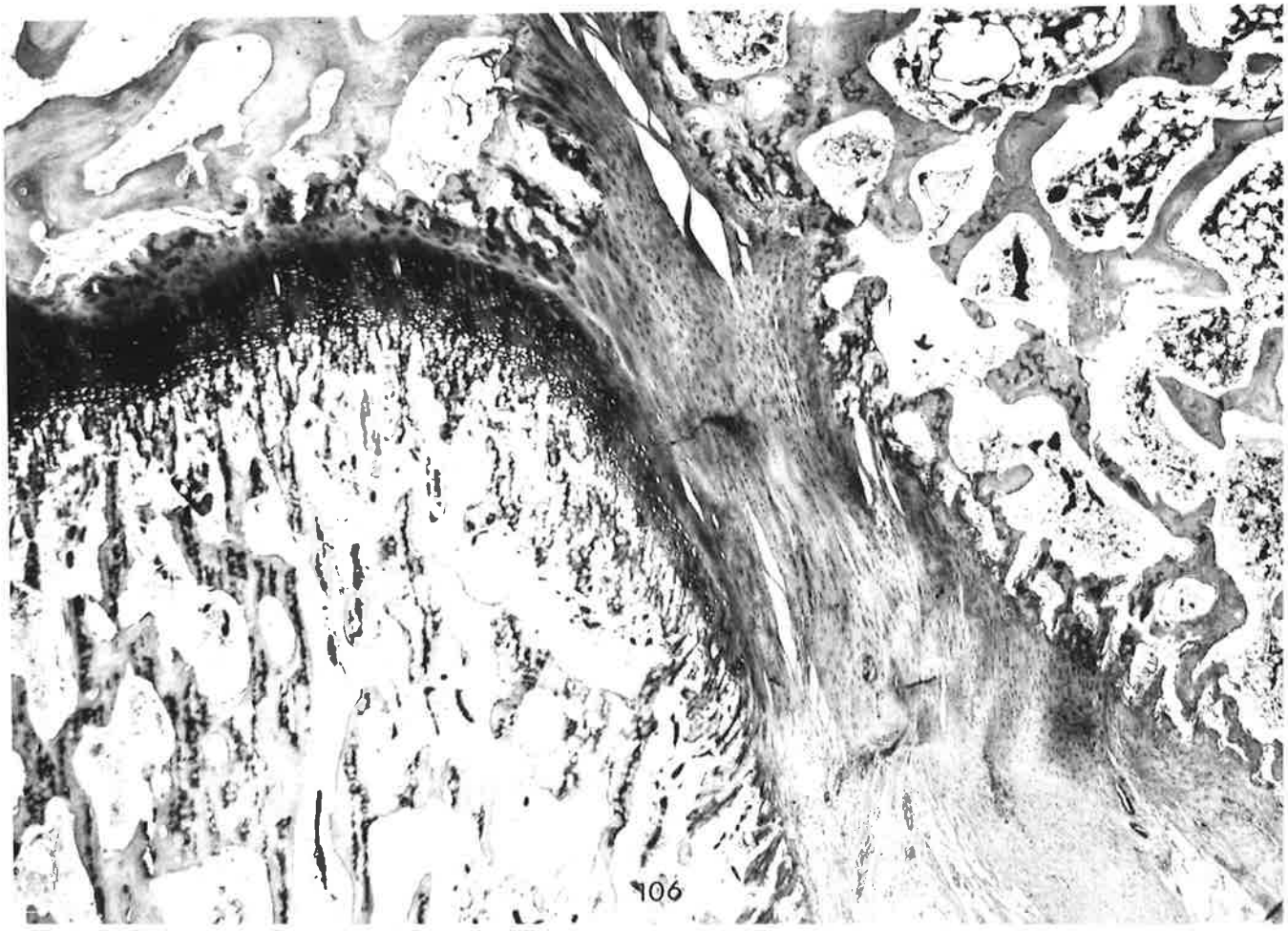
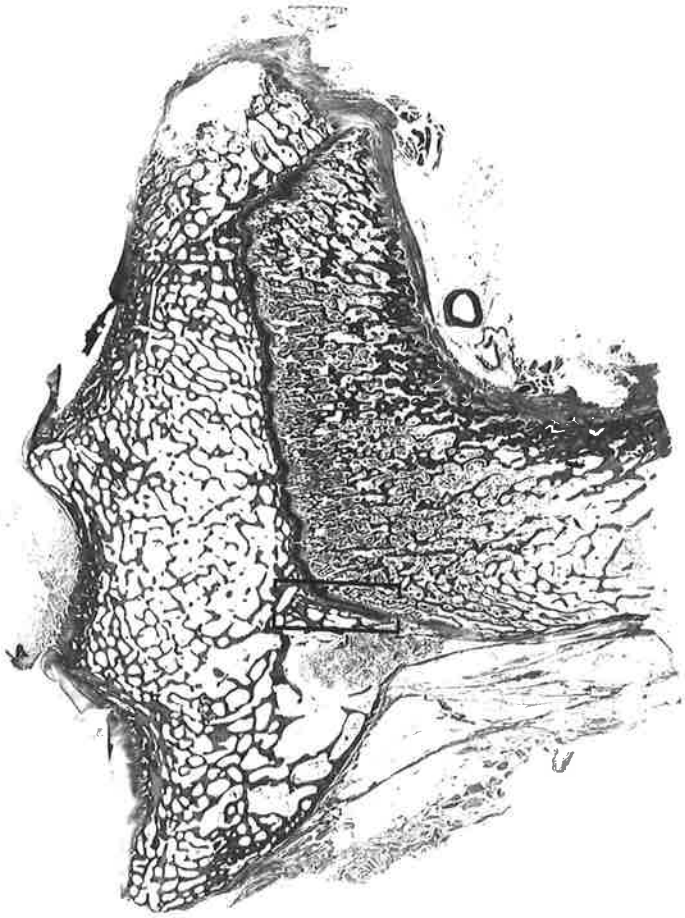
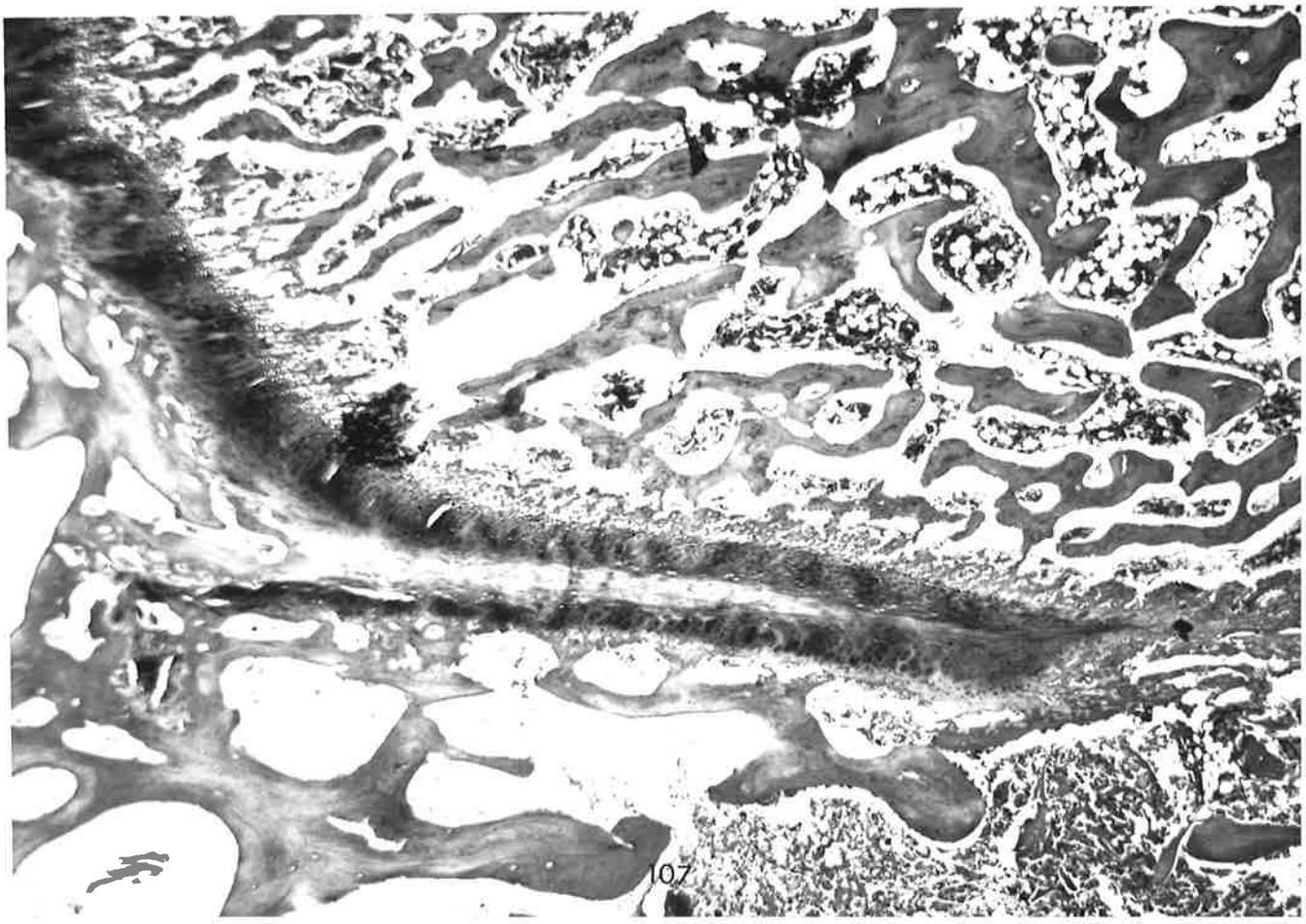


Figure 5.8.3: A complete section of the upper tibia in Animal 18 demonstrates a grade (++) physeal response. (x 2.5)

High power of the inner field demonstrates a bimodal contour to the physeal proliferation. There is loss of normal physeal architecture in contact with the fibrous capsule of the fat graft. (x 150)





Secondly, the proliferation of physeal tissue was examined to evaluate the type of the cartilage. Under polarized light fibrocartilage can be seen separate from the normal physeal cartilage (Fig. 5.9).

Thirdly, additional ectopic physis positioned in the region of the fat graft was identified as either metaphyseal or epiphyseal in situ. The peripheral growth plate response was analysed for normality or hypertrophy (Fig. 5.10).

5.5 RESULTS OF 1 SQ. CM. DEFECTS

In the 1 sq. cm. defect series 20 animals were entered. In a manner similar to the previous experiment, the 4 animals who failed to create a deformity were discarded from further analysis. One anaesthetic death occurred during the transfer of the animal to a C.T. scan. One premature autopsy was the result of a wound infection that continued despite antibiotics being administered for three weeks post operatively. The remaining 14 animals were in two groups. One group of 7 animals was killed at three months from the reversal procedure. The other group of 7 animals was killed at six months.

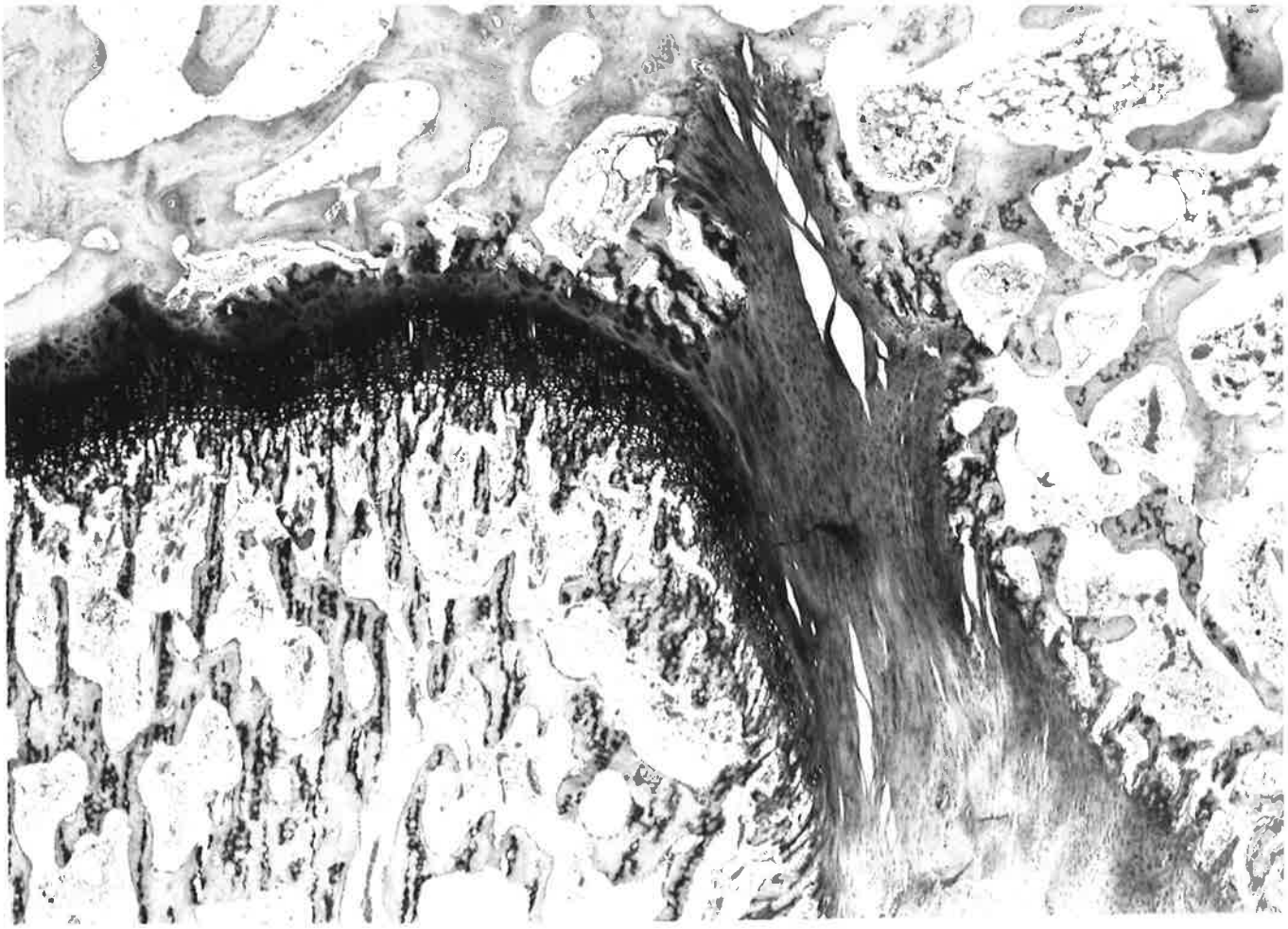
5.5.1 Radiology Results:

For all animals in the 1 sq. cm. group the serial measurements of bone length, pin distance, bone angle, and pin angle are given in Appendix 2. Animals 11-17 had measurements at 6 weeks of age, 18 weeks and at 30 weeks (autopsy). The six-month group (animals 18-24) had measurements at 6 weeks of age, 18 weeks, 30 weeks and at 42 weeks (autopsy).

Figure 5.9: HISTOLOGICAL ASSESSMENT OF THE HOST PHYSEAL RESPONSE - POLARIZED LIGHT EXAMINATION.

Figure 5.9.1: Animal 26 shows grade (++) physeal response with loss of normal cellular organization. (x 325)

Figure 5.9.2: The same section with polarized light. The increased collagen of the cartilage matrix is demonstrated. (x 325)



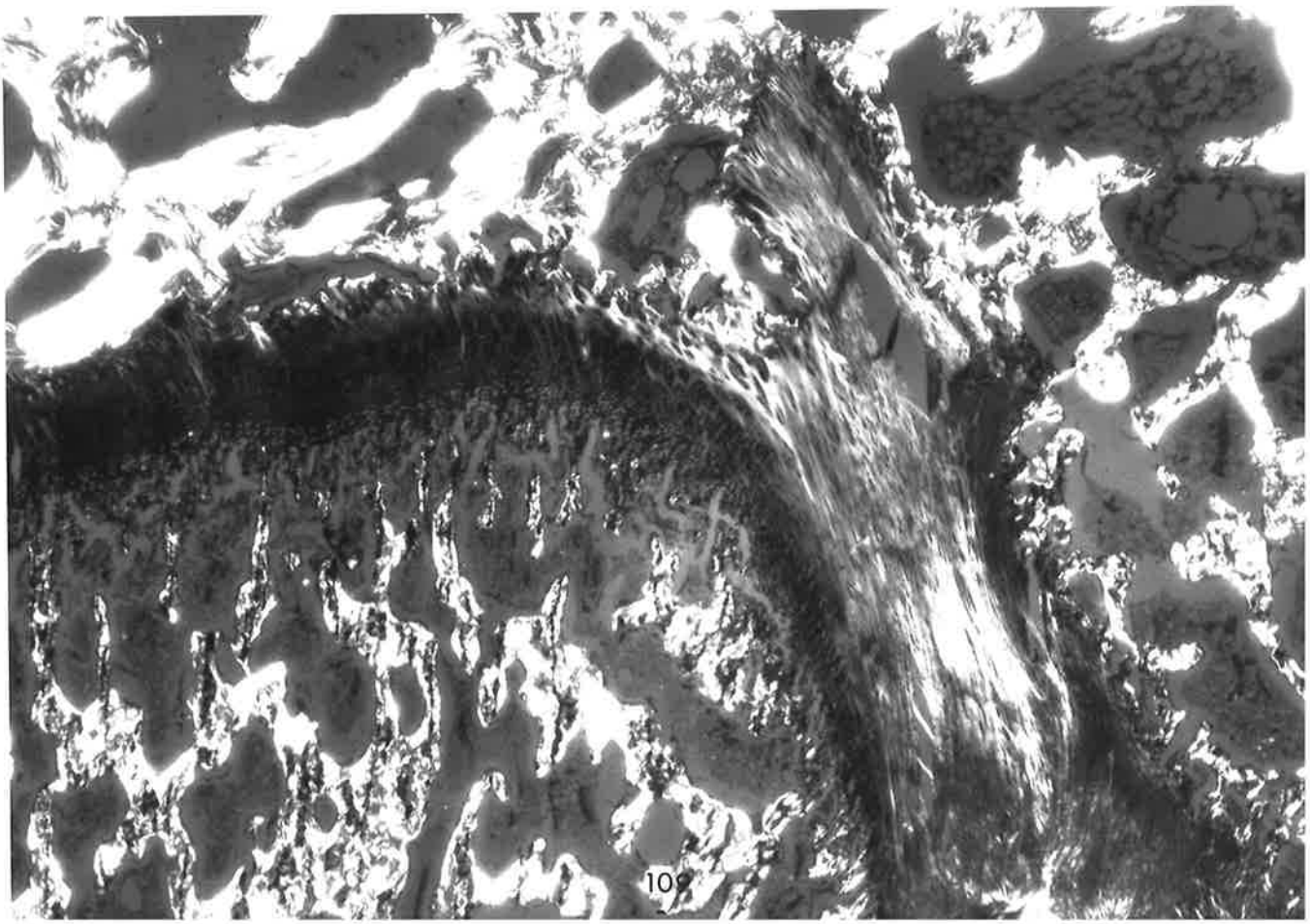
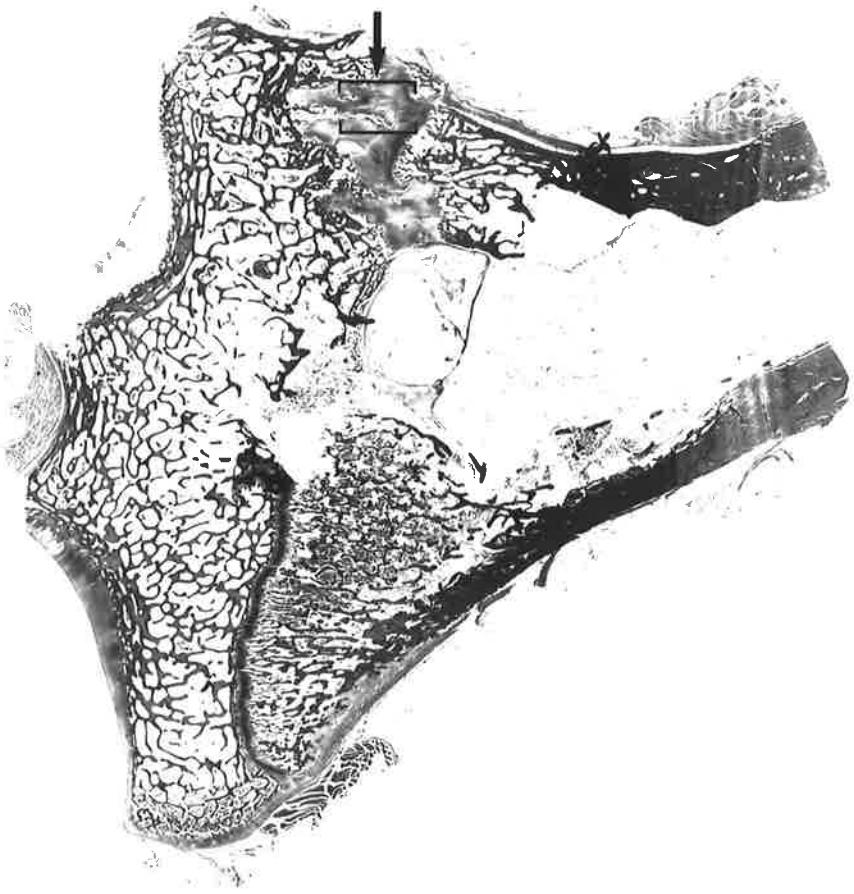
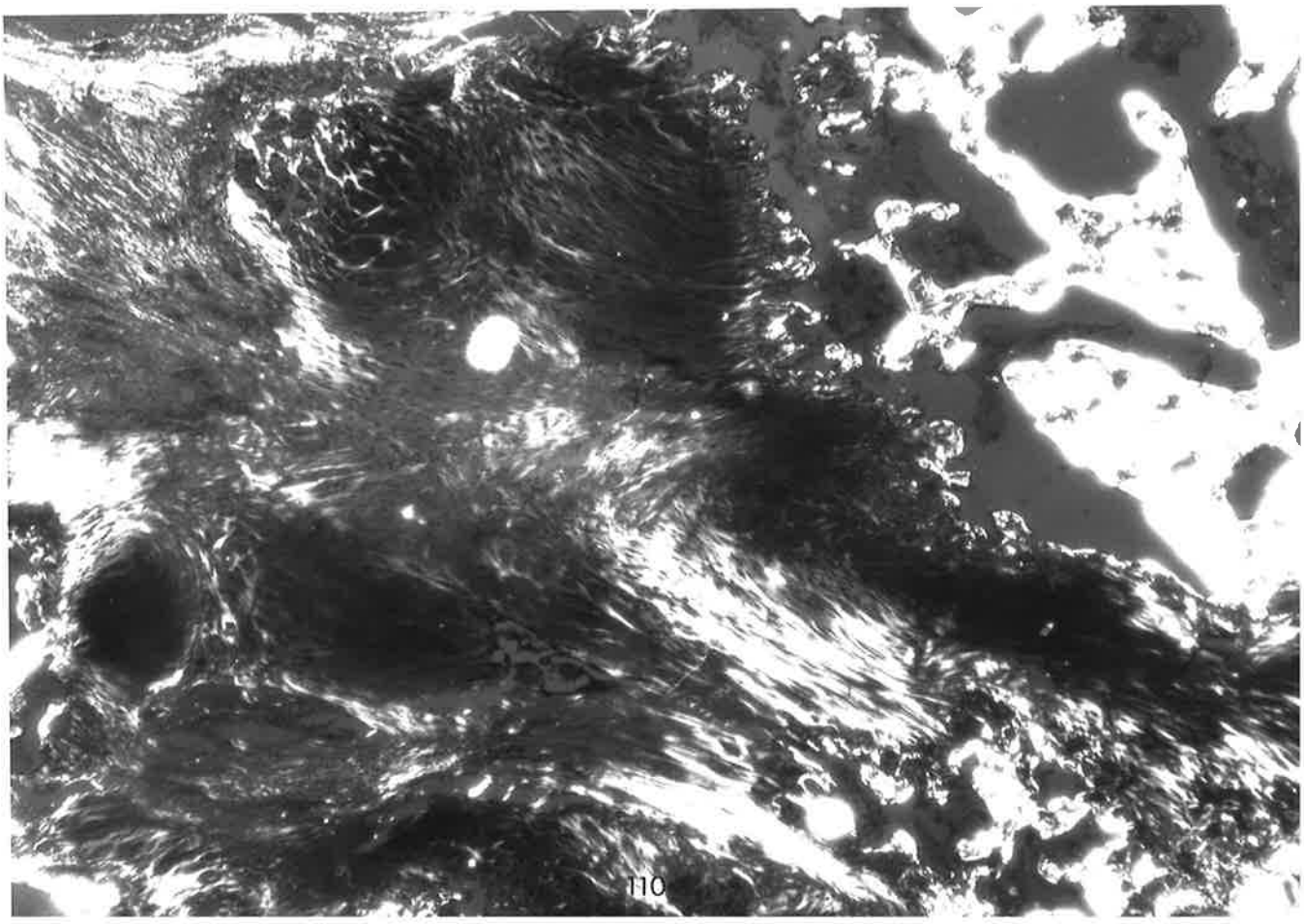


Figure 5.10: **HISTOLOGICAL ASSESSMENT OF THE HOST PHYSEAL RESPONSE - PERIPHERAL PHYSIS.**

Figure 5.10.1: On microscopic examination Animal 37 shows an increase of 3 to 4 times in the width of the peripheral physis. (x 2.5) ➡

Figure 5.10.2: Polarized light examination proves this to be fibrocartilage. (x 325)





The results of the deformity index for 1 sq. cm. defects for three months survival are summarized in Table 5A. All 7 animals in this group produced a bone deformity index that confirmed a medial tibial growth arrest. The mean degree of varus angulation was 22° (range 18-33°) and the bone length ratio decreased from 1.000 to 0.600.

After the reversal procedure at 18 weeks 5 of 7 animals showed a mean of 8.4° (range 6-14°) improvement of the varus angle and concurrent improvement towards 1.000 of the bone length ratio. Two animals, 15 and 16, showed no improvement of either parameter, indicating failure of the reversal procedure.

The results of the deformity index for 1 sq. cm. defects in animals of six months survival are summarized in Table 5B.

In every case varus deformity was produced. The mean degree of varus was of 21° (range 15-27°). The pin angle difference then improved a mean of 8.5° (range 6-11°) in the first three months, and all bone length ratios showed concurrent improvement towards 1.000.

In the next three months the rate of correction of varus averaged 2° (range 0-4°). There was continued improvement of the bone length ratio toward 1.000.

A typical example of improvement is animal 24, in which radiologically a 25° varus deformity improved 6° in the first three months and then 1° in the next three months (Fig. 5.11). The initial bone length ratio of 0.992 decreased to 0.611 then improved to 0.794 at three months and finally to 0.792 at six months.

TABLE 5A

1 SQ. CM. DEFECT

3 MONTH SURVIVAL

DEFORMITY INDEX AT 3 MONTHS

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	($^{A}/_{B}$ ratio)		
11	6	0.136	0.155	0.877		
	18a	0.094	0.216	0.435	33° varus	REVERSAL
Pin reposit.	18b	0.141	0.216	0.653		SUCCESS
	30	0.172	0.228	0.754	6° improved	
12	6	0.148	0.148	1.000		
	18	0.124	0.212	0.584	18° varus	REVERSAL
	30	0.182	0.228	0.798	11° improved	SUCCESS
13	6	0.148	0.158	0.937		
	18	0.133	0.210	0.633	20° varus	REVERSAL
	30	0.188	0.245	0.767	5° improved	SUCCESS
14	6	0.155	0.146	1.062		
	18	0.131	0.192	0.682	19° varus	REVERSAL
	30	0.185	0.209	0.885	14° improved	SUCCESS
15	6	0.147	0.148	0.993		NO
	18	0.128	0.209	0.612	20° varus	REVERSAL
	30	0.128	0.23	0.556	+3° varus worse	FAILURE
16	6	0.148	0.149	0.993		NO
	18	0.116	0.212	0.547	20° varus	REVERSAL
	30	0.122	0.23	0.53	+4° varus worse	FAILURE
17	6	0.135	0.137	0.992		
	18	0.103	0.208	0.495	28° varus	REVERSAL
	30	0.148	0.234	0.632	6° improved	SUCCESS

TABLE 5B

1 SQ. CM. DEFECT

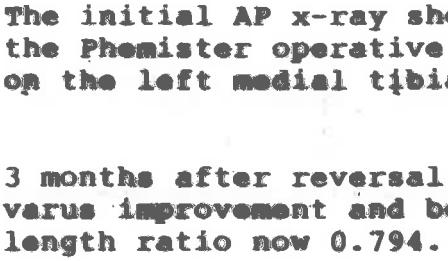
6 MONTH SURVIVAL

DEFORMITY INDEX AT 3 AND 6 MONTHS

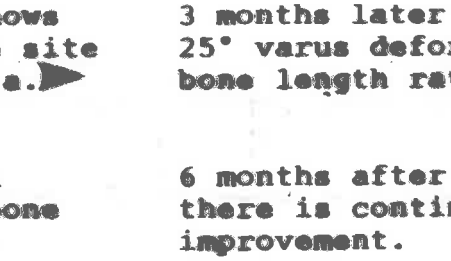
Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	(A/B ratio)		
18	6	0.147	0.148	0.993		
	18	0.123	0.201	0.612	23° varus	REVERSAL
	30	0.184	0.242	0.760	10° improved	SUCCESS
	42	0.236	0.284	0.830	same	
19	6	0.159	0.160	0.994		
	18	0.138	0.199	0.693	21° varus	REVERSAL
	30	0.190	0.223	0.852	8° improved	SUCCESS
	42	0.220	0.261	0.843	3° improved	
20	6	0.153	0.150	1.020		
	18	0.143	0.177	0.807	17° varus	REVERSAL
	30	0.210	0.208	1.010	9° improved	SUCCESS
	42	0.235	0.250	0.940	3° improved	
21	6	0.149	0.149	1.000		
	18	0.141	0.194	0.727	15° varus	REVERSAL
	30	0.198	0.215	0.921	9° improved	SUCCESS
	42	0.232	0.248	0.935	4° improved	
22	6	0.157	0.164	0.957		
	18	0.141	0.222	0.635	19° varus	REVERSAL
	30	0.198	0.254	0.779	11° improved	SUCCESS
	42	0.234	0.275	0.851	1° improved	
23	6	0.141	0.119	1.18		
	18	0.123	0.118	0.654	27° varus	REVERSAL
	30	0.158	0.205	0.77	7° improved	SUCCESS
	42	0.183	0.217	0.843	1° improved	
24	6	0.142	0.143	0.992		
	18	0.118	0.193	0.611	25° varus	REVERSAL
	30	0.173	0.218	0.794	6° improved	SUCCESS
	42	0.19	0.24	0.792	1° improved	

Figure 5.11: **RADIOLOGICAL EVIDENCE OF FUSION AND REVERSAL OF DEFORMITY. (Animal 24)**

The initial AP x-ray shows the Phemister operative site on the left medial tibia. ➤

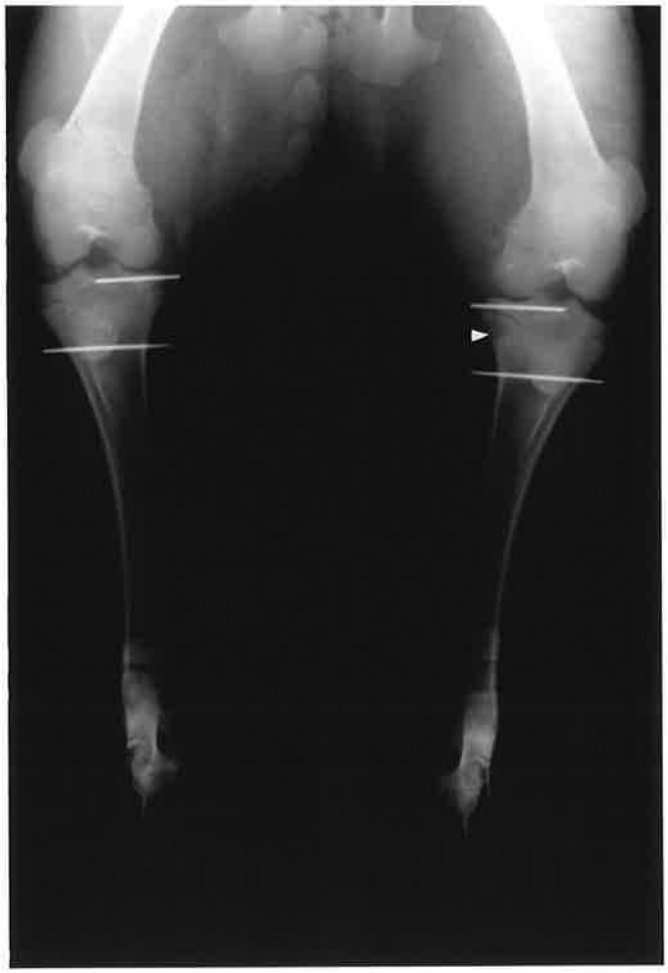


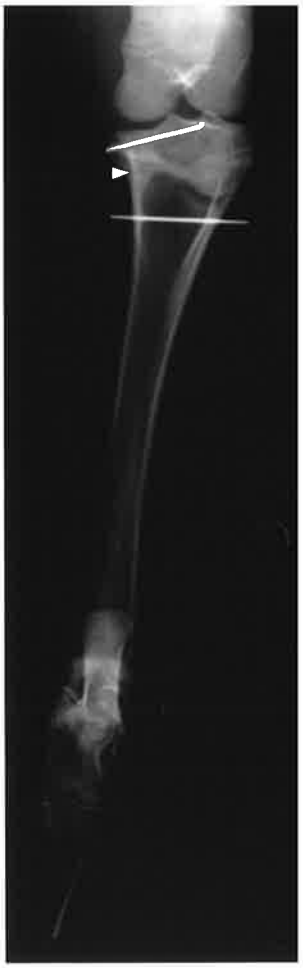
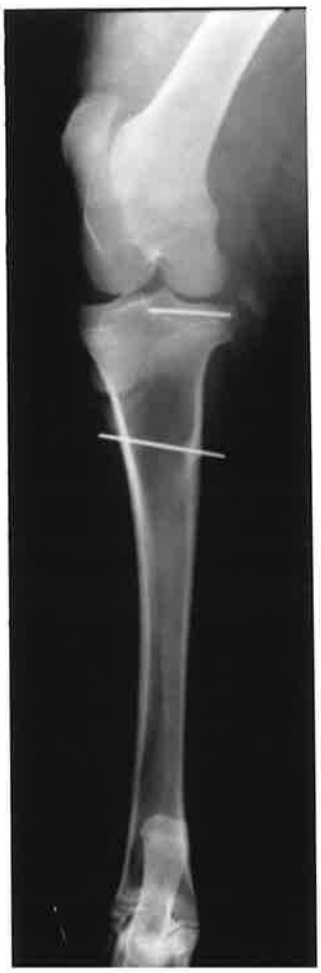
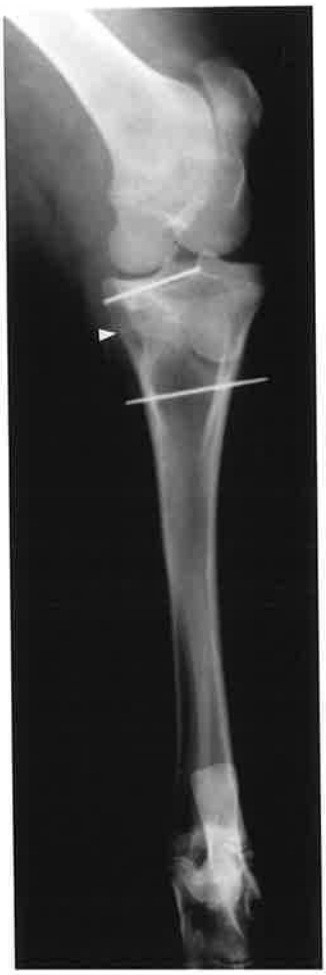
3 months later there is 25° varus deformity. The bone length ratio 0.611.



3 months after reversal varus improvement and bone length ratio now 0.794.

6 months after reversal there is continued improvement.



By radiological criteria the deformity index showed improvement or success in 12 of 14 cases (85.7%).

5.5.2 C.T. Results:

The results of C.T. measurement of the extent of physeal defect at the time of the reversal procedure and at autopsy are seen in Table 5C. The complete table of measurements is given in Appendix 3.

The 7 animals in the three-month autopsy group had a physeal defect at reversal of 17.5 $\pm$ 2.0% (Standard Deviation). The extent of the lesion had increased to 21.3 $\pm$ 4.4% at autopsy ($p=0.1007$, $t=2.1$).

The 7 animals in the six-month autopsy group had a physeal defect at reversal of 16.9 $\pm$ 2.5%. The extent of the lesion had decreased to 15.1 $\pm$ 4.6 at autopsy ($p=0.5034$, $t=0.9$).

There was no statistically significant difference between the initial defect size for the three and six-month groups ($p=0.706$, $t=0.4$).

For 14 animals in this first group the initial size of the defect was 17.2 $\pm$ 2.2%.

5.5.3 Histology Results:

Tables 5D and 5E detail the histological findings.

a. Host Response:

All animals in the three month group showed a (+) or (++) granulation tissue about the fat implant. A pseudo-capsule had

TABLE 5C

C.T. SCAN RESULTS FOR 1 SQ. CM.

AT 3 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment Size
11	17.1	26.5	Increased
12	17.9	21.5	Increased
13	16.6	18.6	Increased
14	15.6	22.4	Increased
15	18.3	20.6	Increased
16	21.2	26.0	Increased
17	15.5	13.6	Decreased
	Mean 17.5 ± 2.0	21.3 ± 4.4	p=0.1007

C.T. SCAN RESULTS FOR 1 SQ. CM.

AT 6 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment Size
18	16.5	17.7	Increased
19	20.9	18.3	Decreased
20	17.5	16.7	Decreased
21	18.8	19.4	Increased
22	17.1	15.6	Decreased
23	13.5	6.4	Decreased
24	14.2	11.7	Decreased
	Mean 16.9 ± 2.5	15.1 ± 4.6	p=0.5034

TABLE 5D

HISTOPATHOLOGY

1 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 3 MONTHS

Animal No.		11	12	13	14	15	16	17
C.T.	1. (I)	17.1%	17.9%	16.6%	15.6%	18.3%	21.2%	15.5%
	2. (F)	26.5%	21.5%	18.6%	22.4%	20.6%	26.0%	13.6%
Fat								
Site		P	P	P	P/M	-	-	P
Viability		N	N	N	Partial Necrosis	Complete Necrosis	Complete Necrosis	N
Fibrosis		+	++	+	++	-	-	+
Host Tissue								
Fibrosis		++	++	+	+	-	-	+
Osteoblasts		+	+	+	++	+++	+++	+
Epiphyseal Bone		++	++	+	++	++	++	++
Cortical		++	+	++	0	0	0	+
Physeal								
Medial Spur	0 Indolent	+	+	+	No	No	++	
Hypertrophy	++	++	+	++	0	0	+	
Peripheral	No	No	No	Yes	No	No	No	
Ectopic	Yes	Yes	No	Yes	No	No	No	
Bone Bridge								
Site	-	-	-	Central Cancellous	Complete Cancellous	Complete Cancellous	No	
Nature				Incomplete	Cortical	Cortical		

TABLE 5E

HISTOPATHOLOGY

1 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 6 MONTHS

Animal No.		18	19	20	21	22	23	24
C.T.	1. (I)	16.5%	20.9%	17.5%	18.8%	17.1%	13.5%	14.2%
	2. (F)	17.7%	18.3%	16.7%	19.4%	15.6%	6.4%	11.7%
Fat								
Site		P	P	P	P	P	P/M	P
Viability		H	H	H	H	H	N	H
Fibrosis		+	+	+	+	0	+/++	+
Host Tissue								
Fibrosis		++	+	+	+	+	+	+/++
Osteoblasts		+	0	0	+	+	+	+
Epiphyseal Bone		+	0	++	+	+	++	+
Cortical		_	+	+	0	+	++	0
Physeal								
Medial Spur		++	++	++	+	+	+	+
Hypertrophy		++	+/++	+	+/++	+	+	+
Peripheral		No	Yes	No	No	No	Yes	No
Ectopic		Yes	No	No	Yes	Yes	No	No
Bone Bridge								
Site		Attempting Central	No	No	No	No	Central	No
Nature		Fibrous					Fibrous	

formed by six months with more specimens showing a (+++) degree of fibrosis. Epiphyseal bone thickening was very marked in 6 of 7 cases and cortical thickening in 4 out of 5 of the three-month group. By six months the cortical bone response was either not present or minimal in 6 of 7 specimens. Additionally, the epiphyseal bone response was present in only 2 of 7 cases.

b. Fat Response:

In 12 of 14 experiments the fat remained at the physis/metaphyseal junction.

Of the 12 animals in which the fat remained at the physis/metaphysis junction the autologous fat graft remained normal in appearance in 4 out of 5 three-month follow up cases. In 6 out of 7 six-month cases there was longitudinal hypertrophy of the fat content. In these cases there was minimal (0 or + grade) fibrosis within the fat. Initially, the fibrosis in the fat was marked (++ grade) in 2 of 5 three-month cases. There was extensive fibrosis in animal 14 where partial necrosis of the fat graft was present.

c. Bone Bridge Reformation:

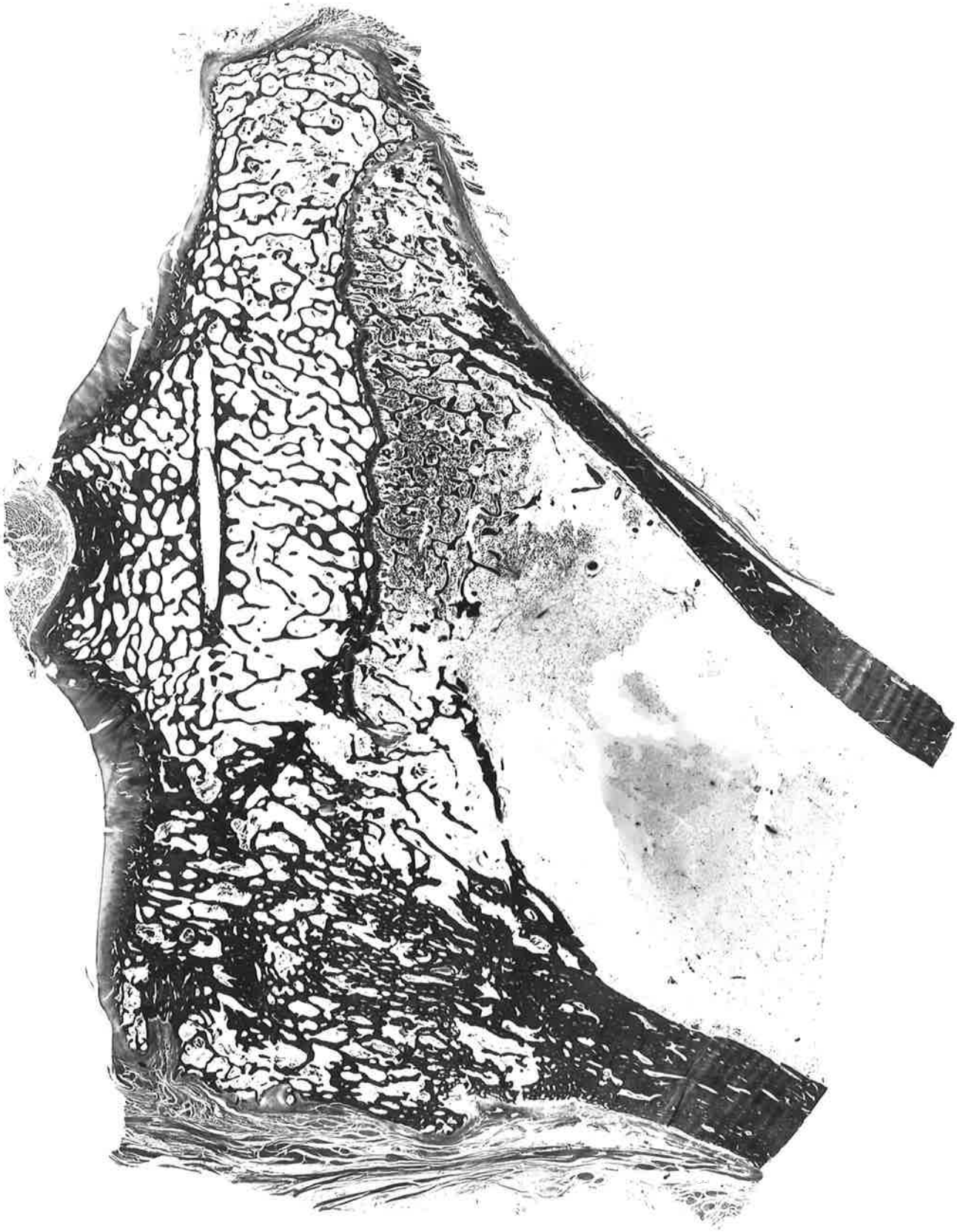
If there is a reformation of the bone bar then continued deformity may occur. In 2 of 7 of the three-month experiments, Animals 15 and 16, there was a complete cancellous/cortical bone bar between the epiphysis and the metaphysis (Fig. 5.12).

Figure 5.12: **BONE BRIDGE REFORMATION - PERIPHERAL.**
(Animal 16)

Either as a result of fat necrosis or loss of position or mal position there is no evidence of fat at the physeal levels.

A complete corticocancellous bar joins the epiphyseal and metaphyseal bone with failure of the reversal procedure.

The findings were similar in Animal 15. (x 5)



In 3 other animals there was a central incomplete lesion: Animal 14 in the three-month group, and Animals 18 and 23 in the six-month group.

d. Physeal Response:

The response of the physis was such that a medial spur of the physeal cartilage was seen extending into the metaphysis in 4 out of 5 three-month cases, and in 7 out of 7, six-month cases.

Hypertrophy in the amount of physeal cartilage medially was most marked in 3 out of 7 six-month cases and in 3 of 5 at three months. Polarized light microscopy revealed that the nature of this tissue was fibrocartilage, and not phenotypical of physeal cartilage in all cases. The degree of disorganization of the physis centrally could produce a bimodal appearance where the convolution of the physis gives the appearance of there being two complete growth plates (Fig. 5.13).

In the two cases (Animals 15 and 16) of complete bone bridge reformation there was evidence of diminution of the height of the physis.

There was marked peripheral proliferation of fibrocartilage in 3 of 12 animals which did not have bone bridge reformation.

Six specimens showed ectopic metaphyseal physeal cartilage.

5.6 RESULTS OF 2 SQ. CM. DEFECTS:

Of the 15 animals in the 2 sq. cm. series 13 animals had reversal operations. There was one anaesthetic death during

Figure 5.13: INTERSTITIAL PHYSEAL REPAIR. (Animal 17)

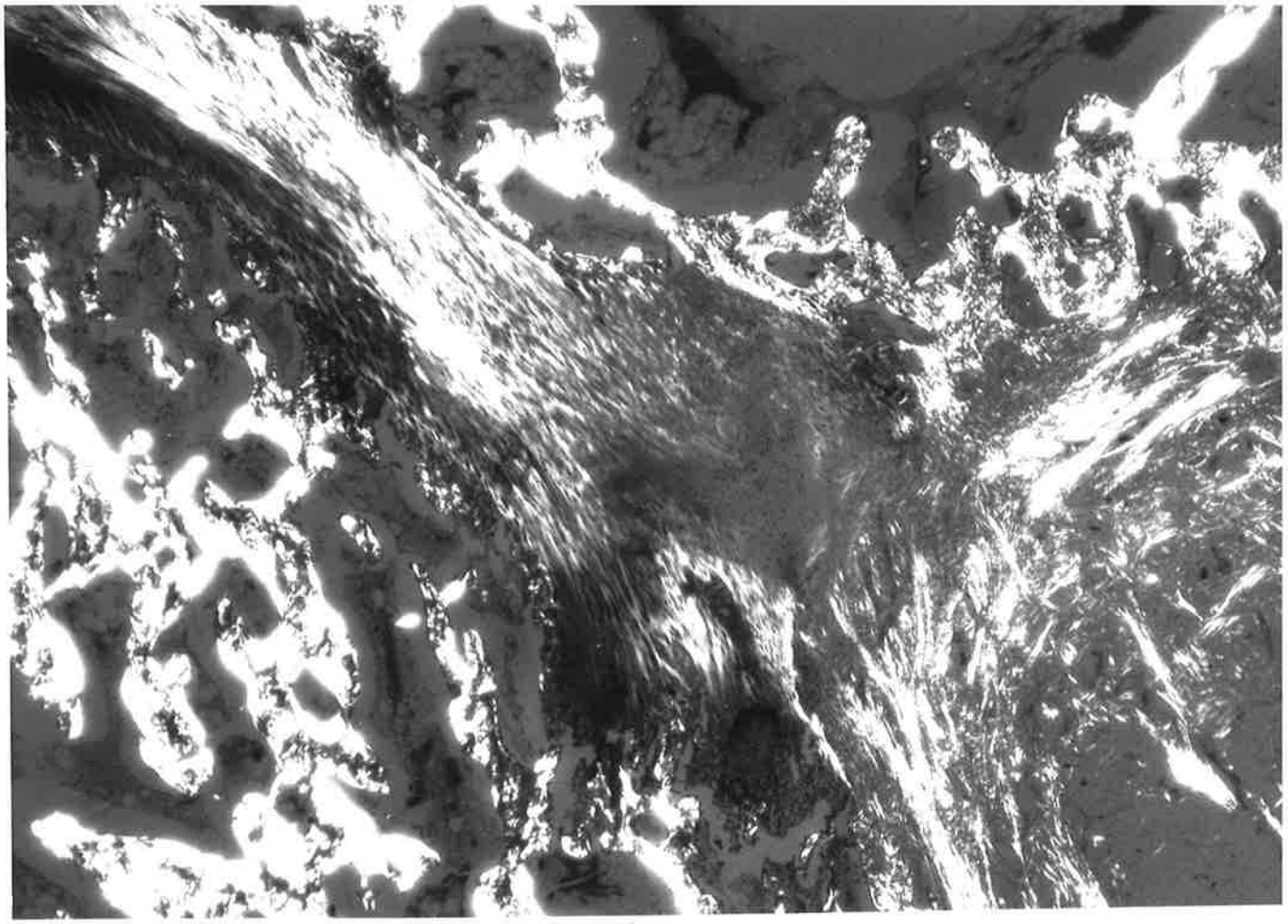
Figure 5.13.1: Immediately adjacent to the fat there is a spur of physeal cartilage that projects into the metaphysis.

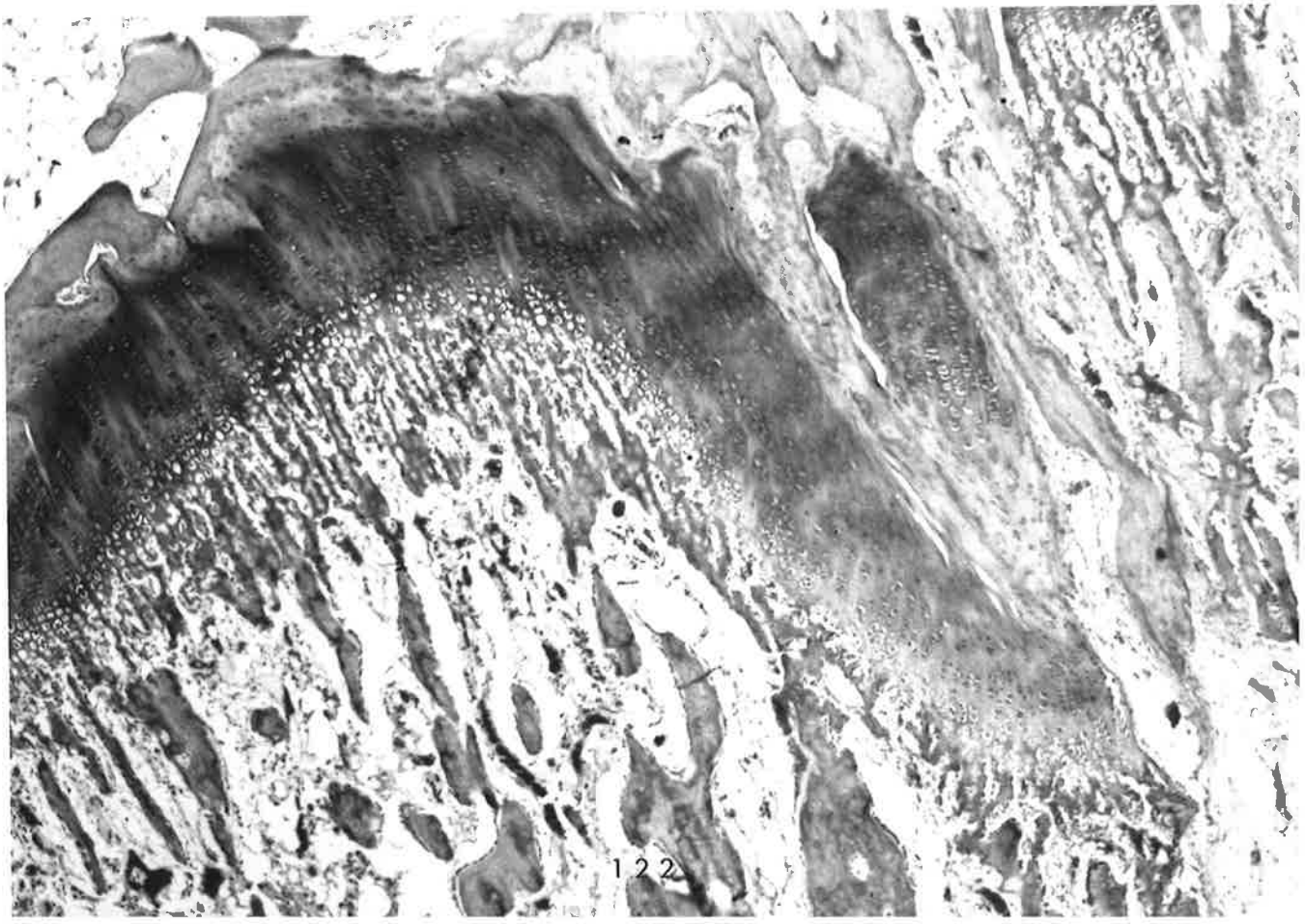
This was present in 11 of 12, 1 sq. cm. defects with a successful reversal outcome.

Hypertrophy of the cartilage shows loss of normal cellular orientation and increased collagen content (polarized light).

Figure 5.13.2: Another section shows an apparent duplication of the growth plate with a bimodal orientation. This represents the increased convolution of the interstitial repair process.

(Similar findings in Animals 18, 19, 20.)





transfer of the animal from the C.T. to the Animal House, and one animal died of tetanus. Eleven animals have complete data. Seven were killed at three months and four at six months.

5.6.1 Radiology Results:

The serial measurements of bone length, pin distance, bone angle, and pin angle are shown in Appendix 4. Animals 25-31 had measurements at 6 weeks of age, 18 weeks and at 30 weeks (autopsy). The six-month group (Animals 32-35) had measurements at 6 weeks of age, 18 weeks, 30 weeks and at 42 weeks.

The results of the deformity index for 2 sq. cm. defects for three-months survival are summarized in Table 5F. All 7 animals in this group produced a bone deformity index that confirmed a medial growth arrest. The mean varus angulation was 21° (range 12° - 37°) and the bone length ratio decreased from 1.000 to the 0.600 level.

After the reversal procedure 3 of 7 showed no worsening of the varus angle. However, only one showed a significant improvement in the varus deformity of 5° and concurrent improvement towards 1.000 of the bone length ratio.

Animals 27, 28, 29, 30 showed no improvement of either parameter thus indicating failure of the reversal procedure.

The results of the deformity index for 2 sq. cm. for six-months survival are summarized in Table 5G.

TABLE 5F

2 SQ. CM. DEFECT

3 MONTH SURVIVAL

DEFORMITY INDEX AT 3 MONTHS

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	(A/B ratio)		
25	6	0.164	0.178	0.921		
	18	0.141	0.238	0.592	18° varus	REVERSAL
	30	0.172	0.255	0.675	5° improved	SUCCESS
26	6	0.164	0.170	0.965		REVERSAL
	18	0.143	0.218	0.656	20° varus	PARTIAL
	30	0.188	0.265	0.709	same	SUCCESS
27	6	0.158	0.155	1.020		NO
	18	0.150	0.203	0.739	16° varus	REVERSAL
	30	0.161	0.272	0.591	8° worse	FAILURE
28	6	0.162	0.162	1.000		NO
	18	0.137	0.228	0.601	28° varus	REVERSAL
	30	0.153	0.267	0.573	3° worse	FAILURE
29	6	0.165	0.159	1.030		NO
	18	0.106	0.198	0.535	37° varus	REVERSAL
	30	0.115	0.235	0.409	3° worse	FAILURE
30	6	0.154	0.161	0.957		NO
	18	0.131	0.211	0.621	18° varus	REVERSAL
	30	0.133	0.240	0.554	10° worse	FAILURE
31	6	0.139	0.140	0.928		REVERSAL
	18	0.118	0.190	0.621	12° varus	PARTIAL
	30	0.131	0.196	0.668	same	SUCCESS

TABLE 5G

2 SQ. CM. DEFECT

6 MONTH SURVIVAL

DEFORMITY INDEX AT 3 AND 6 MONTHS

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	($^{A}/_{B}$ ratio)		
32	6	0.144	0.150	0.960		
	18	0.124	0.193	0.642	17° varus	REVERSAL
	30	0.169	0.233	0.725	7° improved	SUCCESS
	42	0.188	0.243	0.774	2° improved	
33	6	0.146	0.152	0.960		
	18	0.182	0.212	0.858	11° varus	REVERSAL
	30	0.205	0.244	0.840	2° improved	SUCCESS
	52	0.234	0.273	0.857	3° improved	
34	6	0.151	0.151	1.000		NO
	18	0.125	0.202	0.619	25° varus	REVERSAL
	30	0.175	0.246	0.711	1° worse	PARTIAL
	52	0.195	0.272	0.716	4° improved	SUCCESS
35	6	0.161	0.161	1.000		
	18	0.132	0.224	0.589	25° varus	REVERSAL
	30	0.188	0.254	0.740	16° improved	SUCCESS
	52	0.181	0.300	0.603	same	

3 out of 4 six-month animals showed improvement of the indices mostly in the initial three months. By radiological criteria the deformity index showed improvement or success in 6 of 11 cases (54.5%).

5.6.2 C.T. Results:

The results of the C.T. measurement of the extent of the physeal defect at the time of the reversal procedure and at sacrifice are seen in Table 5H. The complete table of measurement is in Appendix 5. For the 11 cases with reversal the initial C.T. measured 28.0 $\pm$ 6.0%.

The 7 animals in the three-month autopsy group had a physeal defect at reversal of 26.3 $\pm$ 5.3%. This had decreased to 25.0 $\pm$ 7.3% at autopsy ($p=0.723$, $t=0.4$).

The 4 animals in the six-month autopsy group had a physeal defect at reversal of 30.8 $\pm$ 6.9%. The extent of the lesion had decreased to 22.4 $\pm$ 3.7% at autopsy. This failed to reach statistical significance ($p=0.1041$, $t=2.1$). There was not a statistically significant difference between the three and six month groups ($p=0.9928$).

5.6.3 Histological Results:

In the 2 sq. cm. series, 7 of the animals were autopsied at three months and 4 at six months (Tables 5I & 5J).

a. Host Response:

As has been noted with the 1 sq. cm. defect series, the host tissue response showed an initial osteoblastic response being +

TABLE 5H

C.T. SCAN RESULTS FOR 2 SQ. CM.

AT 3 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment Size
25	30.6	30.6	Same
26	21.8	23.9	Increased
27	29.3	24.4	Decreased
28	30.2	36.9	Increased
29	18.1	23.5	Increased
30	31.3	22.9	Decreased
31	23.1	13.3	Decreased
	Mean 26.3 ± 5.3	25.0 ± 7.3	p=0.723

C.T. SCAN RESULTS FOR 2 SQ. CM.

AT 6 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment
32	29.8	21.3	Decreased
33	23.0	19.1	Decreased
34	30.7	21.6	Decreased
35	39.8	27.7	Decreased
	Mean 30.8 ± 6.9	22.4 ± 3.7	p=0.104

TABLE 5I

HISTOPATHOLOGY

2 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 3 MONTHS

Animal No.		25	26	27	28	29	30	31
C.T.	1. (I)	30.6%	21.8%	29.3%	30.2%	18.1%	31.3%	23.1%
	2. (F)	30.6%	23.9%	24.4%	36.9%	23.5%	22.9%	13.3%
Fat								
Site		P	P	P	P	P	Was P	P
Viability		N	P/N	N	N	N	C/N	P/N
Fibrosis		0	++	0	0	0	++	++
Host Tissue								
Fibrosis		+	++	+	+	+	++	++
Osteoblasts		+	++	+	+	+	++	++
Epiphyseal Bone		++	+	+	+	++	+	++
Cortical		++	+	+	+/++	++	+	++
Physeal								
Medial Spur		No	++	+	+	+	No	+
Hypertrophy		+	++	+	+	++	+++	+++
Peripheral		Yes	Yes	No	Yes	No	No	No
Ectopic		No	?	Yes (M)	No	No	No	No
Bone Bridge								
Site		No	No	No	No	No	Complete	No
Nature							Cancellous	

TABLE 5J

HISTOPATHOLOGY

2 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 6 MONTHS

Animal No.		32	33	34	35
C.T.	1. (I)	29.8%	23.0%	30.7%	39.8%
	2. (F)	21.3%	19.1%	21.6%	27.7%
Fat					
Site		P	P	P/M	P
Viability		N	H	N	N
Fibrosis		+	0	0	+
Host Tissue					
Fibrosis		++	+	+	+
Osteoblasts		0	0	++	0
Epiphyseal Bone		++	+	+	+
Cortical		++	+	++	++
Physeal					
Medial Spur		+	++	++	++
Hypertrophy		No	++	++	No
Peripheral		No	No	No	No
Ectopic		No	No	No	No
Bone Bridge					
Site		Att.	No	No	Att.
Nature				(At)	

or ++ in all three-month animals, whereas only one six-month animal had this osteoblastic response.

The epiphyseal bone response showed thickening more marked in the three-month animals than the six-month specimens. However, the epiphyseal bone response was always (+) for these larger defects. A plateau fracture of Animal 29 was noted. The width of the epiphyseal bone was always increased either + or ++. The cortical bone thickness was increased in all experiments, and double the cortex width in 4 of 7 three-month cases and 3 of 4 six-month cases.

b. Fat Responses:

In 11 of 11 experiments the fat remained in the physeal/metaphyseal junction. However, in one autograft there was a remnant of fat only at the physeal level with evidence of complete necrosis, and a significant amount of fibrous tissue (Animal 30).

7 out of 7, three-month cases had physeal position at the appropriate level, with 4 showing no necrosis, 2 showing partial necrosis, and one indicating complete necrosis (Animal 30). With complete and partial necrosis there were marked fibrous tissue septae within the graft.

3 out of 4 six-month cases showed normal fat, and one, Animal 33, had hypertrophy of the autograft with an apparent increase in size of the fat tissue. Where the fat was viable, there was always minimal or little tissue fibrosis.

c. Bone Bridge:

Bone bridges were not seen in 6 of 7 three-month specimens, but the specimen from Animal 30 showed a complete cancellous bone bridge with failure of the reversal procedure.

None of the six-month specimens showed a complete bone bridge formation. However, 3 of 4 showed an incomplete bone bridge or attempt at bone bridge formation between the metaphysis and the epiphysis. A micro-fracture effect in Animals 32, 34, and 35 could be demonstrated (Fig. 5.14). There is an incomplete tether to the process of growth. The immature woven bone shows longitudinal array of the bone along lines of tension between the epiphysis and metaphysis (Fig. 5.15).

d. Physeal Response:

The response of the physis showed a medial metaphyseal spur formation in all specimens other than in Animal 30 where there was a complete bone bridge reformation. The medial physeal response was ++ in 3 out of 4 of the six-month animals.

There was hypertrophy of the physeal cartilage with disorganization in 4 out of 7 three-month animals. This hypertrophy was not found in 2 out of 4 of the six-month animals.

Peripheral physeal enlargement was seen in 3 of the 11 animals. This was noted only in the three-month specimens. This hyperplasia of the zone of Ranvier cartilage, was also associated with the presence of ectopic sites of physeal

Figure 5.14: **ATTEMPT AT MEDIAL BONE BRIDGE REFORMATION - LOW POWER.**

This whole section of tibia from Animal 35 demonstrates the stable fat graft in position and apparent widening of the epiphyseal bone.

Immediately adjacent to the physeal repair there is an attempt at a cancellous bar formation.* (x 4)

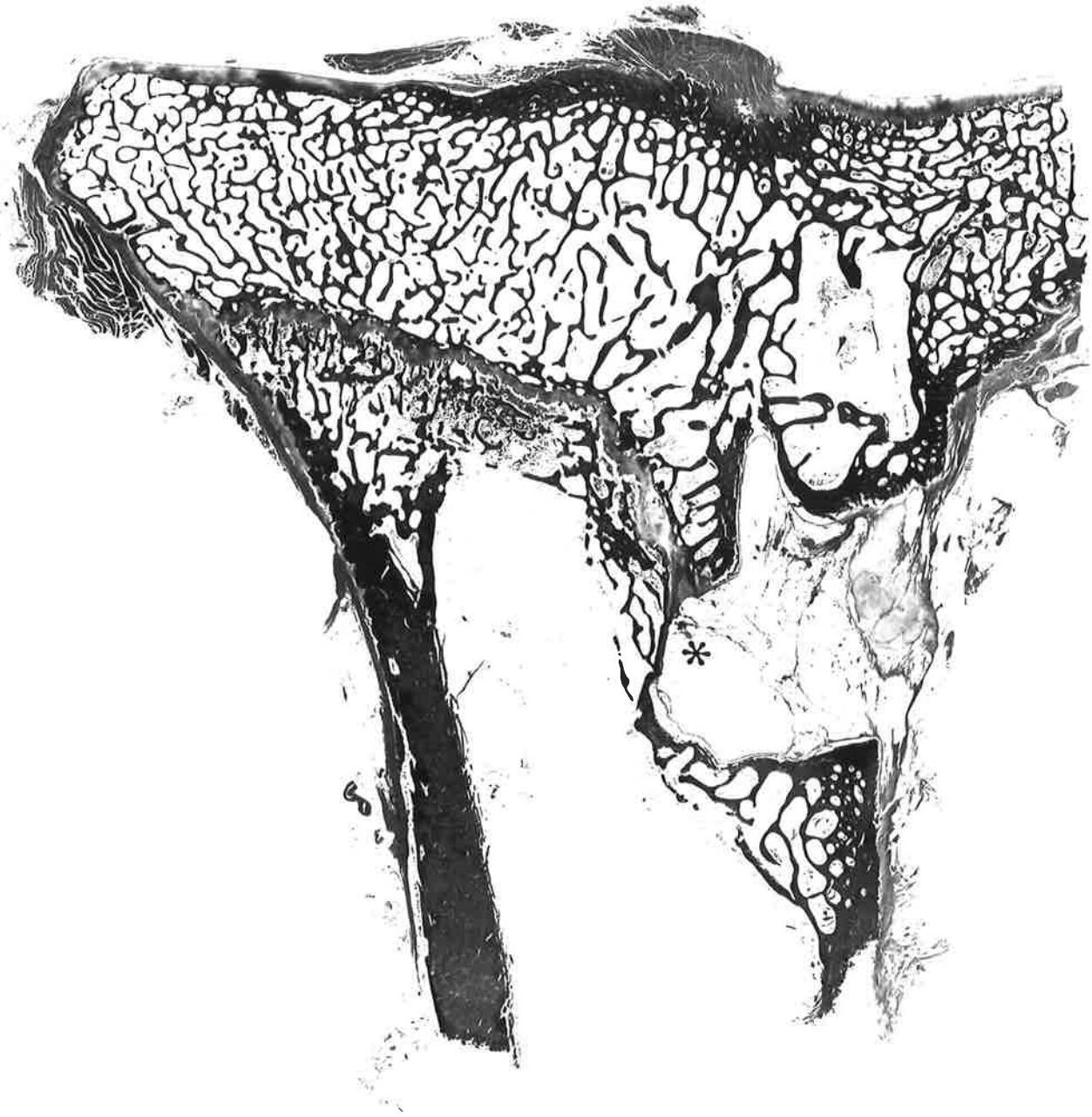


Figure 5.15: **ATTEMPT AT MEDIAL BONE BRIDGE REFORMATION.** (Animal 35) (x 350)

The immature woven bone shows longitudinal array of the bone between the epiphysis and metaphysis along lines of tension (growth) immediately adjacent to the physis.

This "micro-fracture" effect has also been noted in Animals 32, 34.

cartilage as for example in a specimen from Animal 27. Here, metaphyseal cartilage rests were seen.

5.7 RESULTS OF 3 SQ. CM. DEFECTS:

Of the 10 animals with a 3 sq. cm. defect one animal did not have a reversal procedure as part of documentation of the natural history study. One animal died as a result of misadventure. There were thus 8 animals with the largest defects that have complete data. Five were killed at three months and 3 at six months.

5.7.1 Radiological Results:

The serial measurements of bone length, pin distance, bone angle and pin angle are shown in Appendix 6. Animals 36-40 had measurements at 6 weeks of age, 18 weeks and at 30 weeks (autopsy).

The six-month group (Animals 41-43) had measurements at 6 weeks of age, 18 weeks, 30 weeks and at 42 weeks.

The results of the deformity index for 3 sq. cm. defects at three months survival are summarized in Table 5K.

Only 1 of the 5 three-month animals showed improvement of the degree of varus. Of the other four animals two were respectively 7° and 1° worse. Two showed no change in the varus angle. Where the degree of varus remained the same or improved, the bone length ratio improved towards 1.000. For example, Animal 39, in which the bone length ratio at reversal was 0.550 and a 13° varus angle showed no angular change, but the ratio improved to 0.640°. Hence where there was

TABLE 5K

3 SQ. CM. DEFECT

3 MONTH SURVIVAL

DEFORMITY INDEX AT 3 MONTHS

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	($^{A}/_{B}$ ratio)		
36	6	0.142	0.145	0.979		NO REVERSAL FAILURE
	18	0.096	0.209	0.459	28° varus	
	30	0.076	0.256	0.297	7° worse	
37	6	0.190	0.157	1.21		PARTIAL SUCCESS
	18	0.135	0.232	0.581	17° varus	
	30	0.154	0.258	0.600	same	
38	6	0.143	0.135	0.135		NO REVERSAL FAILURE
	18	0.103	0.201	0.201	24° varus	
	30	0.116	0.206	0.206	1° worse	
39	6	0.128	0.147	1.870		PARTIAL SUCCESS
	18	0.111	0.203	0.550	13° varus	
	30	0.133	0.208	0.640	same	
40	6	0.122	0.120	1.02		REVERSAL SUCCESS
	18	0.078	0.178	0.270	12° varus	
	30	0.102	0.183	0.557	4° improved	

improvement or no change in angle, but the bone length ratio showed that there was some resumption of longitudinal growth; these have been determined to be evidence of a partially successful result (Table 5K).

The results of the deformity index for 3 sq. cm. defects at six months survival are summarized in Table 5L. In this group of animals, 2 of 3 showed improvement initially, with one of these animals then failing to sustain the improvement. The other animal (Animal 43) showed progressive failure of radiological bone deformity indices.

The success of the operation of fat interposition by radiological criteria was 4/8 or 50%.

5.7.2 C.T. Results:

In Table 5M the C.T. results show that the mean of the initial defect was 34.2% $\pm$ 8.4% for the three-month group. Although 4 of 5 cases showed a decrease in area, there was not a statistically significant difference between the initial C.T. and the C.T. at autopsy, where the mean was 34.7% $\pm$ 8.1% for three months ($p=0.1625$, $t=1.8$).

The six-month animals had an initial defect size of 32.7 $\pm$ 6.3%. The final area was 36.2 $\pm$ 9.6%. There was not a statistically significant difference in the defect size ($p=0.6354$, $t=0.5$). The mean defect size initially for the 8 animals in the 3 sq. cm. group was 33.7 $\pm$ 7.2%.

TABLE 5L

3 SQ. CM. DEFECT

6 MONTH SURVIVAL

DEFORMITY INDEX AT 3 AND 6 MONTHS

Animal No.	Age (weeks)	Pin Distance Bone Length Ratio		Bone Length ratio	Pin Angulation Difference	Comment
		Operated A	Control B	($^{A}/_{B}$ ratio)		
41	6	0.139	0.139	1.000		
	18	0.126	0.198	0.636	20° varus	REVERSAL
	30	0.172	0.243	0.708	4° improved	SUCCESS
	42	0.177	0.242	0.736	2° improved	
42	6	0.146	0.152	0.961		NO
	18	0.128	0.206	0.621	14° varus	REVERSAL
	30	0.191	0.246	0.776	14° improved	INITIAL
	42	0.194	0.250	0.776	1° worse	SUCCESS
43	6	0.158	0.154	1.02		
	18	0.139	0.206	0.675	30° varus	NO
	30	0.128	0.23	0.556	3° worse	REVERSAL
	42	0.148	0.247	0.599	2° worse	FAILURE

TABLE 5M

C.T. SCAN RESULTS FOR 3 SQ. CM.

AT 3 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment Size
36	35.8	24.6	Decreased
37	47.6	37.8	Decreased
38	25.2	15.8	Decreased
39	13.6	24.0	Increased
40	30.9	21.5	Decreased
	Mean 34.2 ± 8.4	34.7 ± 8.1	p=0.163

C.T. SCAN RESULTS FOR 3 SQ. CM.

AT 6 MONTH SURVIVAL

Animal No.	Initial C.T. (%)	C.T. at Sacrifice (%)	Comment
41	27.3	30.0	Increased
42	31.2	31.4	Same
43	39.7	47.2	Increased
	Mean 32.7 ± 6.3	36.2 ± 9.6	p=0.635

5.7.3 Histological Results:

In the 3 sq. cm. series 5 of the animals were autopsied at three months and 3 at six months (Table 5N, 5O).

a. Host Response:

The host tissue showed the features as previously described in the 1 sq. cm. and 2 sq. cm. series. There was initial increase in cortical thickness and osteoblastic activity in the epiphyseal bone noted in all cases. At the six month review there was evidence of a plateau fracture in one animal (Animal 41).

b. Fat Response:

In all 8 experiments the fat remained at the physeal level and there was no necrosis of the fat. 4 of 8 animals had minimal evidence of interstitial fibrosis.

c. Bone Bridge:

Bone bridge formation was present in 4 of 8 specimens. There were 2 peripheral cortical bars, and one (Animal 36) additionally had a cancellous central bar (Fig. 5.16). There were 2 other incomplete cancellous central bars. One animal (Animal 39) showed a micro fracture effect of this bone bridge as had been previously noted. This animal was autopsied at three months (Fig. 5.17).

d. Physeal Response:

The physeal response showed a medial spur in all 8 specimens. There was hypertrophy in 3 of 3, six-month cases, and 3 of 5

TABLE 5N

HISTOPATHOLOGY

3 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 3 MONTHS

Animal No.		36	37	38	39	40
C.T.	1. (I)	35.8%	47.6%	25.2%	31.6%	30.9%
	2. (F)	24.6%	37.8%	15.8%	24.0%	21.5%
Fat						
Site		P	P	P	P	P
Viability		N	N	N	N	N
Fibrosis		0	+	0	0	+
Host Tissue						
Fibrosis		0	0	0	++	0
Osteoblasts		0	0	0(+in Peri)	++(Peri)	++(Peri)
Epiph. Bone		++	+	++	+	++
Cortical		++	+	N/A unable to classify	+	+
Physeal						
Medial Spur		+	+	+	+	+
Hypertrophy		No	No	+	+	+
Peripheral		No	Yes	No	Yes	Yes
Ectopic		No	No	No	No	No
Bone Bridge						
Site		C + P	No	Peripheral	Attempt at Peripheral	No
Nature		Medially-Can Periph.-Cort.		Cortical	Cancellous	

TABLE 50

HISTOPATHOLOGY

3 SQ. CM. REVERSALS

SHEEP SACRIFICED AT 6 MONTHS

Animal No.		41	42	43
C.T.	1. (I)	27.3%	31.2%	39.7%
	2. (F)	30.0%	31.4%	47.2%
Fat				
Site		P	P	P
Viability		N	N	N
Fibrosis		+	+	0
Host Tissue				
Fibrosis		++	+	0
Osteoblasts		0	0	0
Peiph. Bone		+	+++	+
Cortical		++	++	+
Physeal				
Medial Spur		+	++	+
Hypertrophy		+	+	++
Peripheral		No	No	No
Ectopic		No	No	No
Bone Bridge				
Site		small CENTRAL attempting	No	No
Nature		medially cancellous		

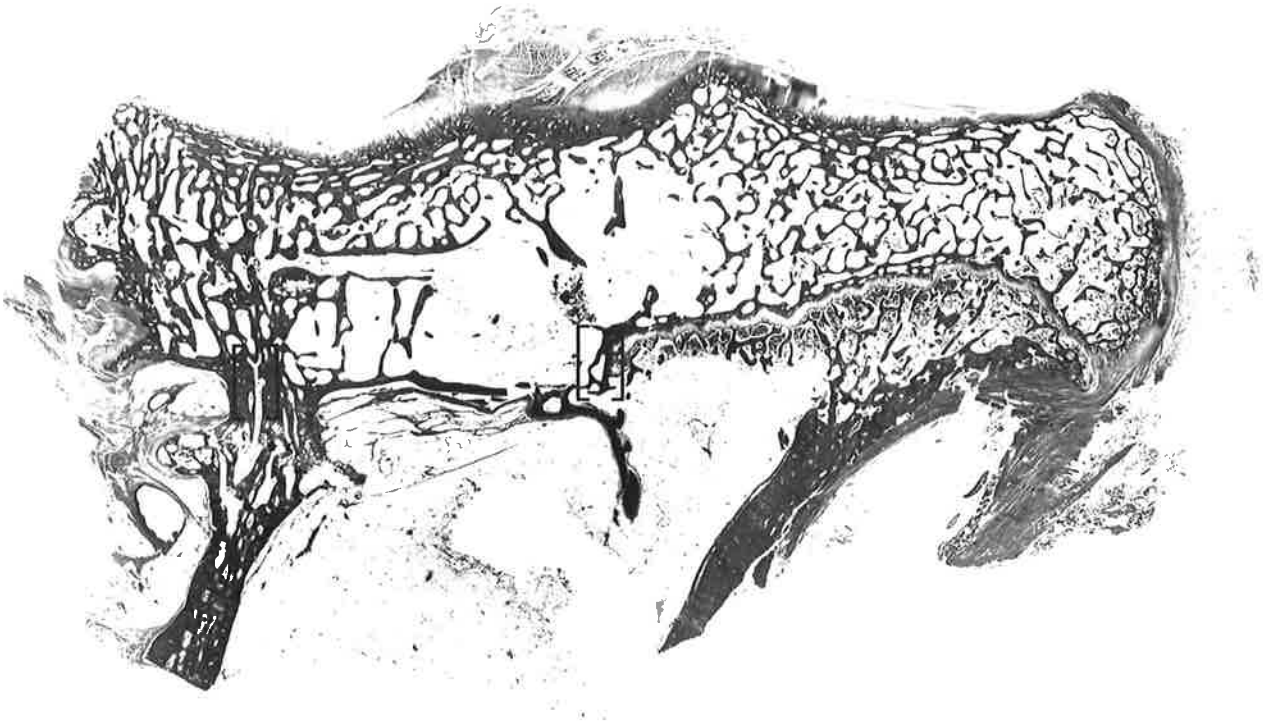
Figure 5.16: **BONE BRIDGE REFORMATION I.**

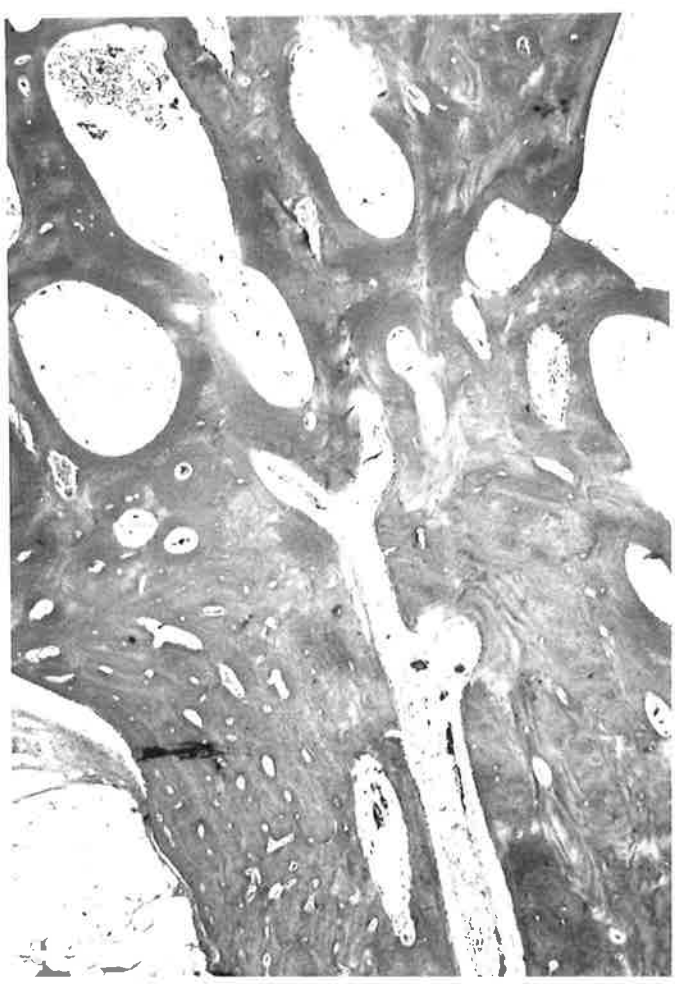
Animal 36 shows a peripheral and a central bone bar recurrence. There is deformity evident from the slide with failure of the reversal procedure despite obvious fat viability. (x 3.5)

High power view shows complete bridges without fracture.

(i) peripherally
(x 325)

(ii) centrally
(x 325)





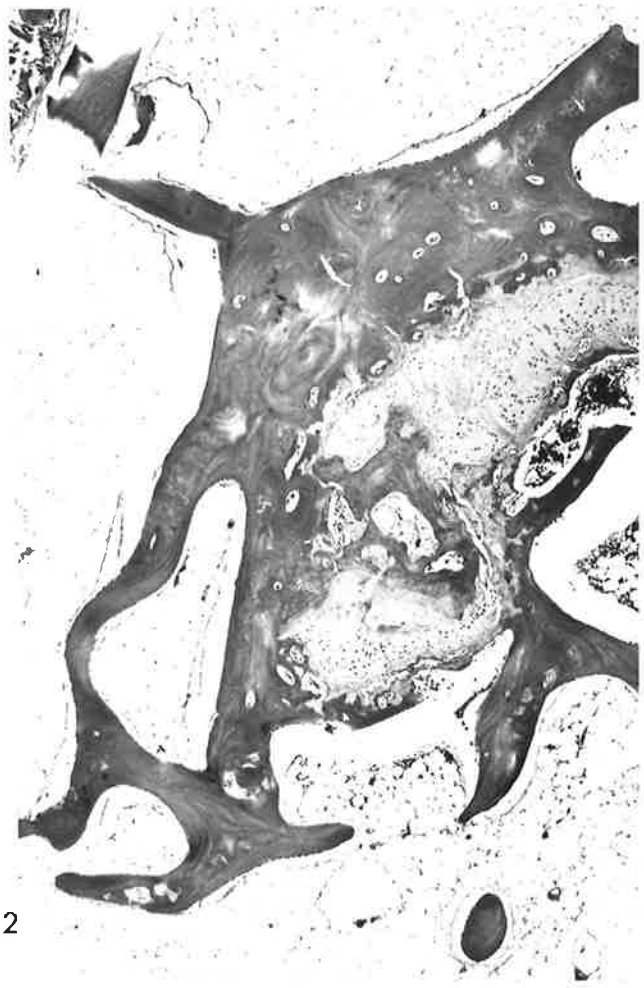
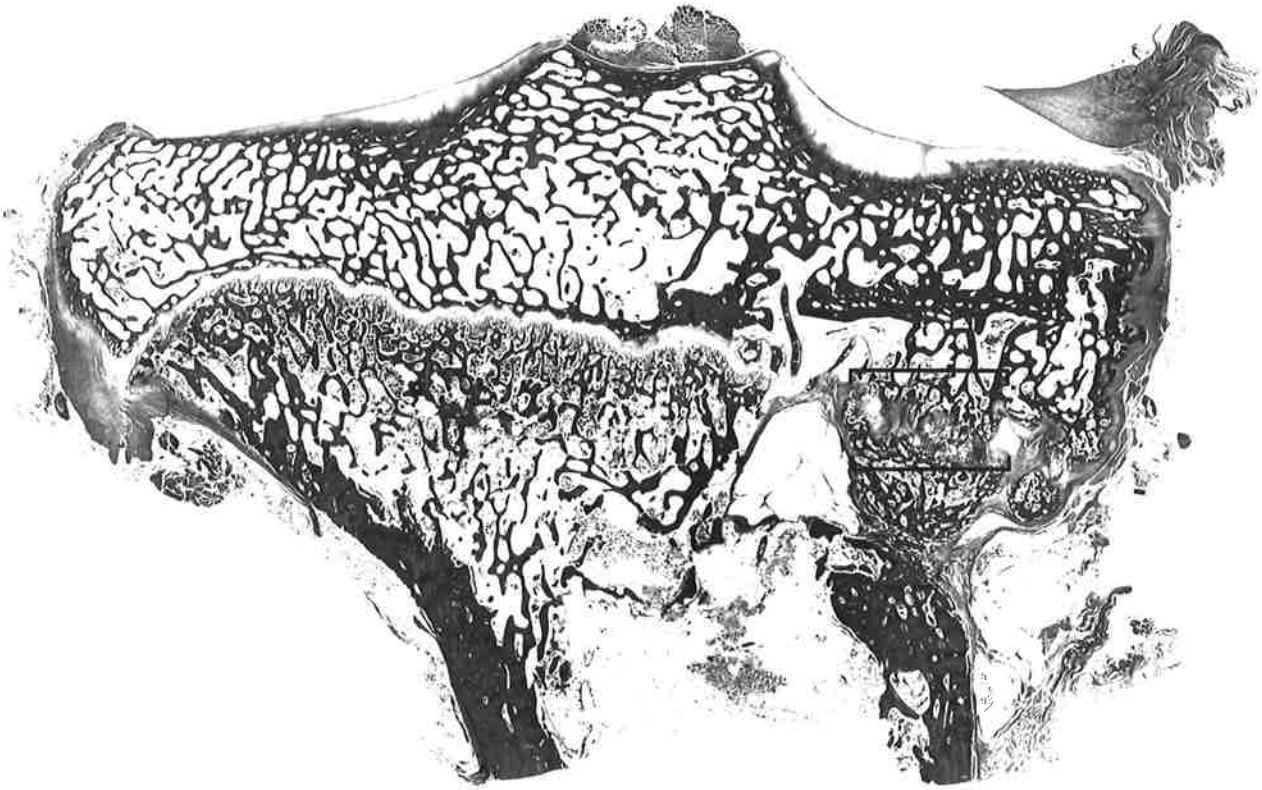
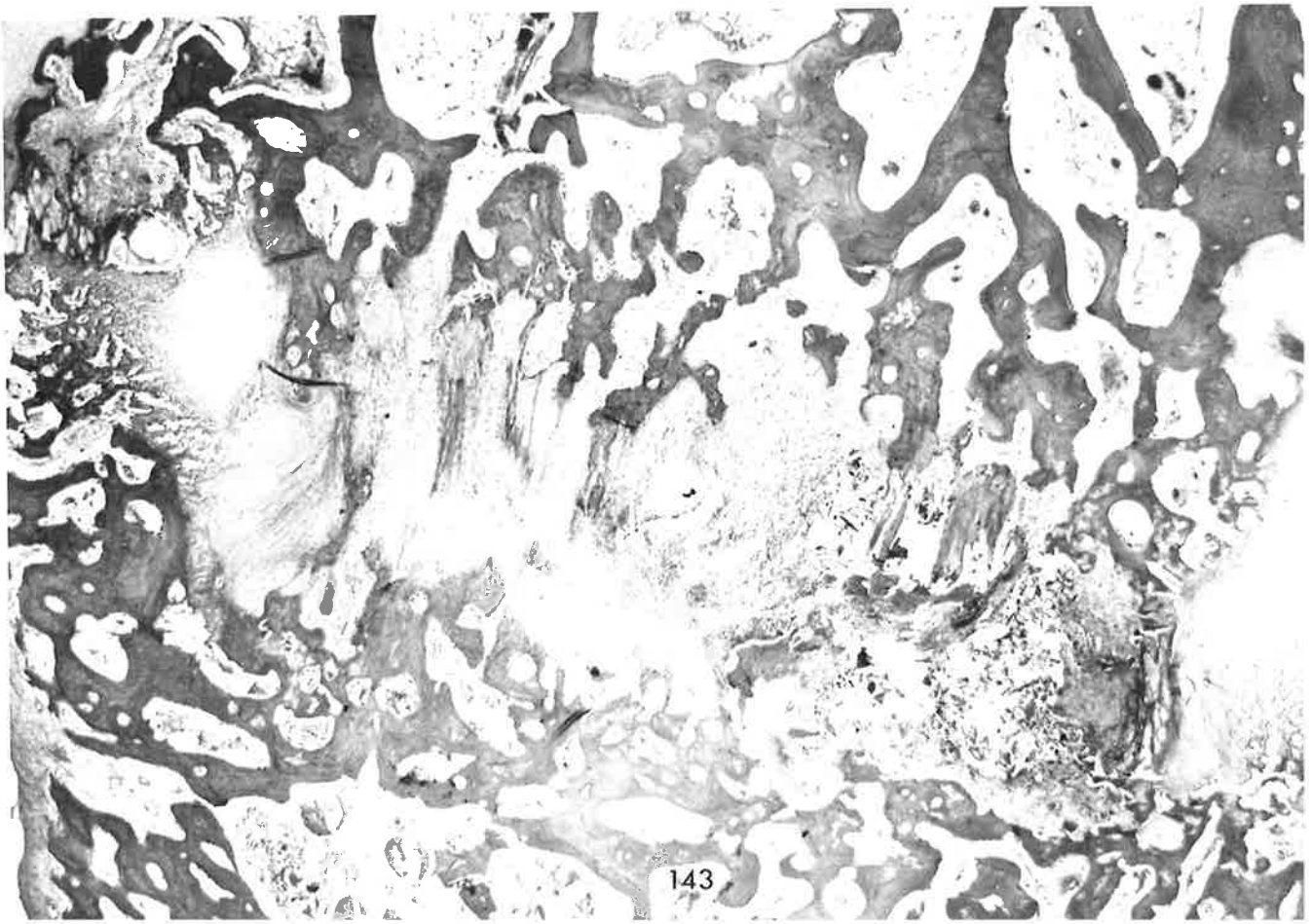


Figure 5.17: BONE BRIDGE REFORMATION II.

Animal 39 had a viable fat graft and an attempt at bone bridge reformation peripherally. (x 3.5)

The high power view shows the immature woven bone with fibrous tissue of a fracture. (x 325)





three-month animals. One of the animals without hypertrophy of the plate had a bone bridge reformation. There were no ectopic rests of cells. 3 of 5 three-month animals (Animals 37, 39 and 40), showed a marked peripheral physeal proliferation.

Chapter 6 DISCUSSION

By comparing the results of the 3 groups of animals the incidence of success, and the causes of failure of fat as an interpositional graft material, can be defined.

In the 1 sq. cm. defect animals the radiological deformity showed that 12 of 14 (85.7%) animals had improvement of varus. The improvement of varus also correlated with an improvement of the bone length ratio towards 1.000.

This improvement was most noticeable at three months, but the effect continued up to six months. However, the rate of improvement decreased. Animal 22 is an excellent example of this phenomenon where the varus of 19° improved by 11° in the first three months, and then only a further 1° in the subsequent three months. The bone length ratio showed improvements from 0.654 to 0.770 at three months and finally 0.843 at six months.

In the 2 sq. cm. defect group 6 of 11 animals (54.5%), showed improvement of the varus deformity. The rate of improvement showed the same trend as in the smaller defect group, with improvement most marked in the first three months. Animal 32 shows this effect where the initial varus of 17° improved by 7° in the first three months, and only 2° in the next three months. The bone length ratio also reflected a successful reversal procedure. In one animal (Animal 26), where angulation remained the same, the ratio improved from 0.656 to 0.709 indicating some continued longitudinal growth.

In the 3 sq. cm. defect group only 4 of 8 cases (50.0%) showed radiological improvement. In this group the degree of angulation improvement was much less noticeable. For example Animal 41 showed 4° improvement initially, and 2° subsequent improvement. Two animals showed no varus angulation improvement, but improvement of the bone length ratio towards 1.000. The degree of improvement, and also the rate of improvement, was decreased with the larger growth plate resection.

The principal cause of failure in the first series of animals was determined by either the failure of survival of fat or the inability to maintain the fat in position. Because haemostasis is not guaranteed, a process similar to fracture repair is not inhibited, and this results, as in Animals 15 and 16, with a bone bridge reformation.

The initial C.T. scan area for the 1 sq. cm. series was 17.2 $\pm$ 2.2%. The initial C.T. area for the 2 sq. cm. series was 28.0 $\pm$ 6.0%. The initial C.T. area for the 3 sq. cm. lesion was 33.7 $\pm$ 7.2%. There was a statistically significant increase in the size of the defect between these groups of animals (p values were < 0.0001). Therefore, the size of the fat graft to fill the cavity was also increased. This may affect the survival of the fat. In the 2 sq. cm. series two animals showed partial necrosis, and one showed complete necrosis: in this animal a cancellous bone bridge did occur with recurrence of deformity (Animal 30).

The bone bridges may be incomplete (Animals 32, and 35) or complete (Animal 38). There may also be a combination of both central and peripheral recurrence (Animal 36).

For all cases the medial cancellous bone bridges show an incomplete bone trabecular formation between the metaphysis and the epiphysis. This may be interpreted as a micro-fracture effect as noted in animals where the provocation operation failed to cause a cessation of growth. Johnson & Southwick (1960) and others have commented upon this. It is evidence of the ability of the physis to maintain growth. This effect is confirmed in the author's series of experiments.

The reasons for failure of fat as the interpositional material are thus:

(i) fat necrosis with failure of revascularization
(ii) fat malposition
(iii) failure of maintenance of position.

If the fat remains viable and in position, what are the factors that determine the host bone and physeal repair processes?

From the histological analysis the host response to the physeal defect can be examined in regions. The epiphysis responds with increased width. There is also an increase in the number of bony trabeculae acting to stress shield the defect in accordance with Wolffs' law (1892). If the defect is large enough (as in Animal 41) then a plateau fracture may occur.

The initial osteoblastic response seen in all 19 three-month specimens becomes much less marked with maturation and remodelling of the surrounding epiphyseal and metaphyseal bone. Initially the epiphyseal bone thickening was ++ or + in 6 of 7 with 1 sq. cm. defects, 7 of 7 with 2 sq. cm. defects and 5 of 5 with 3 sq. cm. defects. At six months only 2 of the 14 animals showed epiphyseal

trabecular thickening, and osteoblastic activity was diminished. Cortical bone thickening was consistent with the response of bone to load in varus. It also remodelled in accordance with Wolffs' law.

Concerning the fat graft at the bone/fat interface: the granulation tissue that would have surrounded the fat soon after implantation gradually showed maturation into fibrous encapsulation of the fat graft. The thickened fibrous tissue at three months had become more mature by six months. The degree of fibrosis depended upon the fat viability. Partial necrosis and septae within the fat graft gave a more marked fibrous response and this fibrous tissue may act as a tether and inhibit normal physeal growth.

With a viable fat material, a stable cavity can be maintained within the bone. It does not heal as would a fracture.

Can the physis respond with an interstitial repair process?

It has been shown that the physis may expand either in a circumferential (diametric) or longitudinal fashion. Diametric expansion as described by Hert (1972) occurs by cell division and matrix expansion within the peripheral physis (interstitial growth).

Tonna (1961), Solomon (1966) and Shapiro (1977) have documented that cellular addition at the physis periphery occurs at the zone of Ranvier. This is described by these authors as appositional growth and may aid in the physeal repair process.

From experimental and clinical evidence Langenskiöld and co-workers have confirmed that central interstitial regeneration of the growth plate occurs [Langenksiöld 1967, 1975, 1978, 1979, 1981, 1986, 1987].

Other experiments in small mammals such as the rabbit demonstrate that partial physeal regeneration follows incomplete and complete physeal/epiphyseal resection [Heikel 1960, 1961, Österman 1972].

However, Haas in 1931, and Banks and Compere in 1941, have been unable to show in experimental studies on rats and rabbits any regenerative potential of the physis. Hert in 1972, also in rabbits was unable to document any central physeal interstitial repair mechanism. He did characterize the peripheral physeal interstitial repair process.

In 1962 Calandruccio found that articular and epiphyseal cartilages of the femoral condyles of skeletally immature dogs were capable of proliferative regeneration and even complete repair with true hyaline cartilage. The observed differences in the repair of incomplete and complete defects suggested that granulation tissue from the subchondral bone of the epiphyseal ossification centre inhibited cartilage formation. If this initial cellular inflammatory response could be prevented or minimized, (i.e. by accurate anatomical reduction), the full potential of cartilage repair might be realized.

Calandruccio noted that cartilage proliferation was indicated by the presence of "chondrons", which were cartilage lacunae with multiple nuclei. Chondron formation was a slow process, and could occur only in the absence of granulation tissue. Such granulation was neither primarily chondrogenic nor disposed to form cartilage secondarily, except perhaps by gradual fibrocartilaginous metaplasia. Calandruccio was the first to suggest a role for the cartilage canal vessels in the chondro-osseous healing process. Wherever the cartilage canal vessels were present, capillary proliferation was evident and formation of granulation tissue occurred.

Consistently in lamb experiments there are central metaphyseal spurs of cartilage. These represent the interstitial repair process. In all cases there is loss of columnar orientation and cartilage proliferation with the formation of cartilage lacunae with multiple nuclei (chondrons). There is a metaplasia of the physeal cartilage to fibrocartilage as proven with polarized light examination. It protrudes into the metaphysis for a variable extent but is always present in some degree (Fig. 6.1).

The fibrocartilage shows increased collagen fibre patterns with a longitudinal orientation towards the metaphysis. Polarization shows that there is an increase in the matrix/cell ratio. The orientation of the collagen is consistent with the normal method of soft tissue/chondro-osseous continuity which allows attachments to grow as the bone undergoes elongation. The metaphyseal direction of the spur of fibrocartilage is consistent with the concept of a metaphyseal tether of the physis which grows away from the point of injury towards the joint surface.

The metaplasia of physeal cartilage can be readily characterized at the edge of the growth plate defect. In Animal 17 the third deeper cut histological section of the tibial specimen shows at the edge of the defect widening and marked physeal metaplasia (Fig. 6.2).

For practical purposes in the larger mammal, sheep, interstitial regeneration with hyaline cartilage does not exist. This finding confirming the views of Haas (1931), Banks and Compere (1941) and Hert (1972).

The periphery of the physis may contribute to the repair process [Keith 1920, Solomon 1966, Shapiro 1977]. In this group of experiments a markedly disorganized cartilage was often noted. The

Figure 6.1: PHYSEAL DEFECT.

Animal 17 shows a viable fat graft at the physeal level with a medial physeal spur.* The host tissue response provides a stable bone defect for the physiological non-union to enable longitudinal growth to proceed. (x 5)

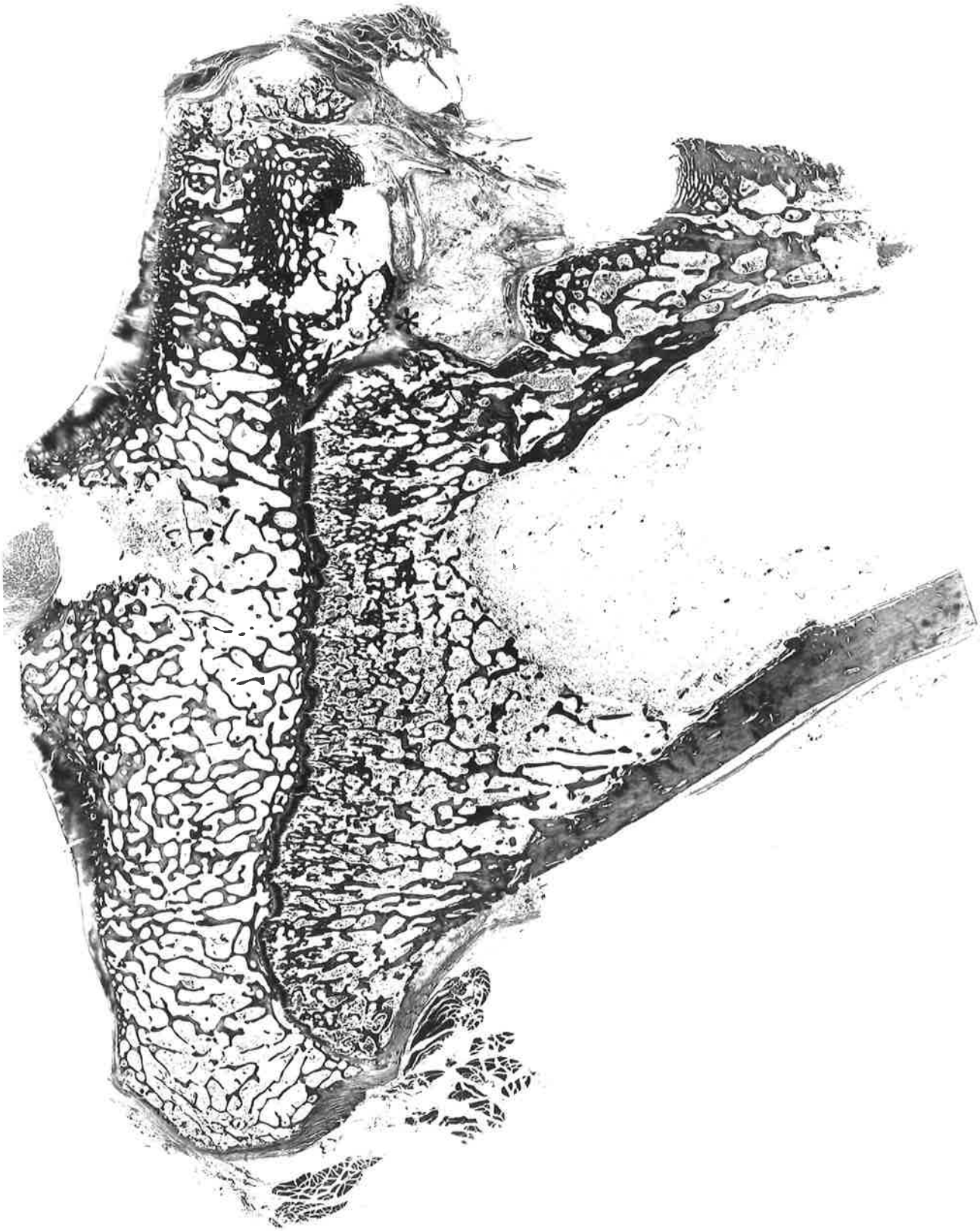
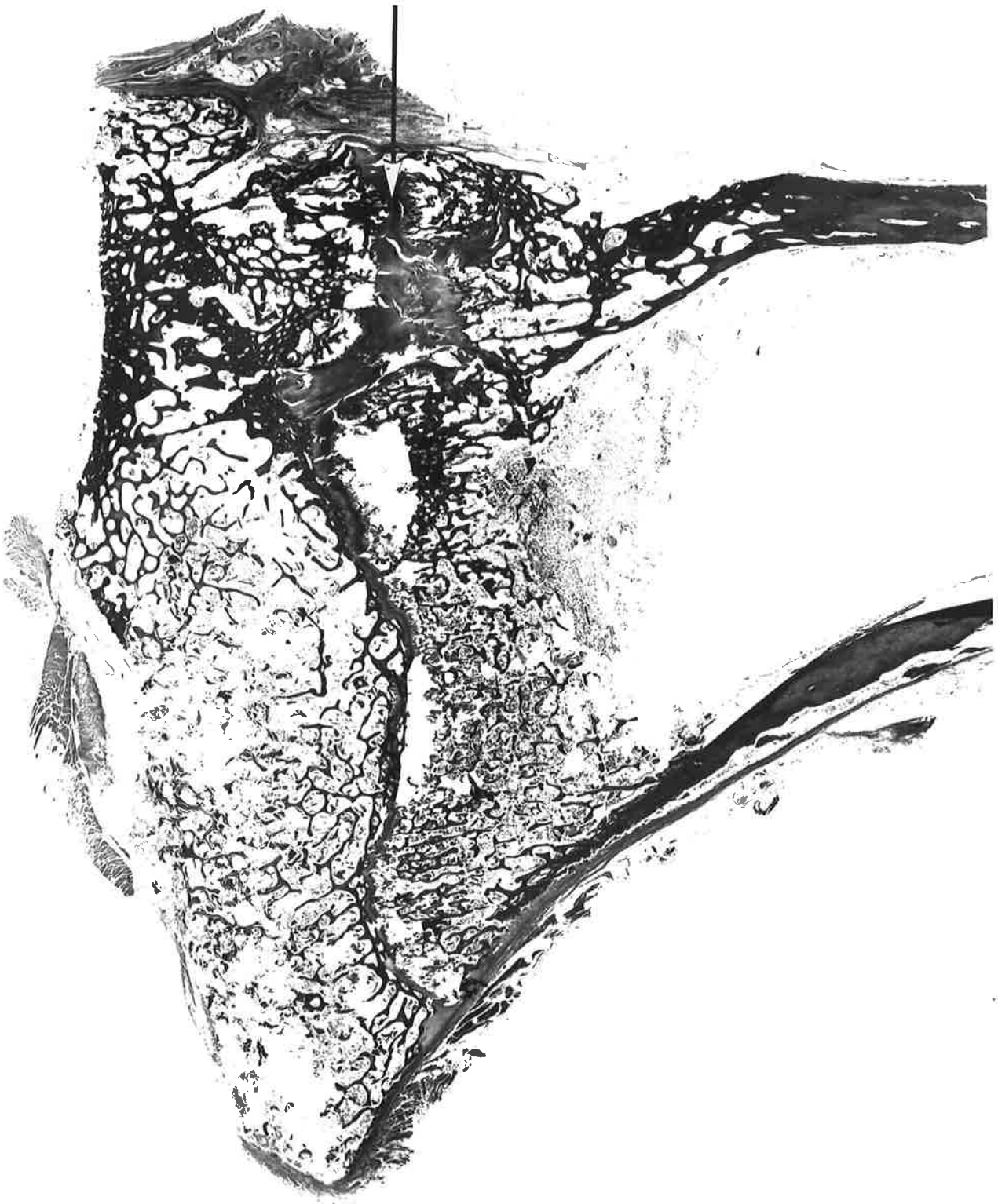


Figure 6.2: PHYSEAL REPAIR

At the edge of the defect in Animal 17 a deeper section indicates the defect margin and response of fibro-cartilaginous metaplasia of the physis. ⟶

The histological evidence is that this represents de-differentiation of the hyaline cartilage as the mechanism of repair. (x 5)



cells for this repair may originate from the Ranvier Zone. Review of the histological work of Österman (1972) and Langenskiöld, Videman, Nevalainen (1986), suggest that the main growth plate "repair" observed in the rabbit model occurred as a result of the ability of these pluripotential cells to grow interstitially. In fact comment is made on an unexplained step-cut peripheral lesion. In these sheep experiments to prevent the formation of a peripheral bone bridge the periosteum was excised, and these cells were not available to be part of the repair process.

An additional series of experiments [Foster, 1984, 1986] was undertaken to investigate the effect of physeal distraction on the process of repair.

Ring (1958), Hert (1969), Ilizarov (1969), Sledge & Noble (1978), Monticelli & Spinelli (1981*a*), De Bastiani (1986), De Pablos (1986), and others had suggested that at a slow rate of distraction physeal replication can occur in accordance with Heuter-Volkmann (1862) principles.

In 14 animals there was a 20 kg. distraction force. A further 7 animals completed low tension distraction of 5 kgs. In both series elongation of the bone averaged 11.0 mm. and maintenance of fat at the defect was consistently noted. Physeal stimulation was noted only in the low tension group with a fracture being produced in the high tension group. These animals had a fracture at different levels within the growth plate which would have led to premature physeal closure. All of the 7 low tension animals showed an increased width of growth plate contour with a stretch of the physis up to twice its width. The tension process did not however increase the interstitial

physeal regeneration to repair the fat interpositional space. Further work on an ideal tension for distraction is required.

The remainder of the physis maintains longitudinal growth providing either:

(i) a unilateral tether can be overcome. That is if a bone bridge does not form or a fracture of the bone bridge occurs (Fig. 6.3).

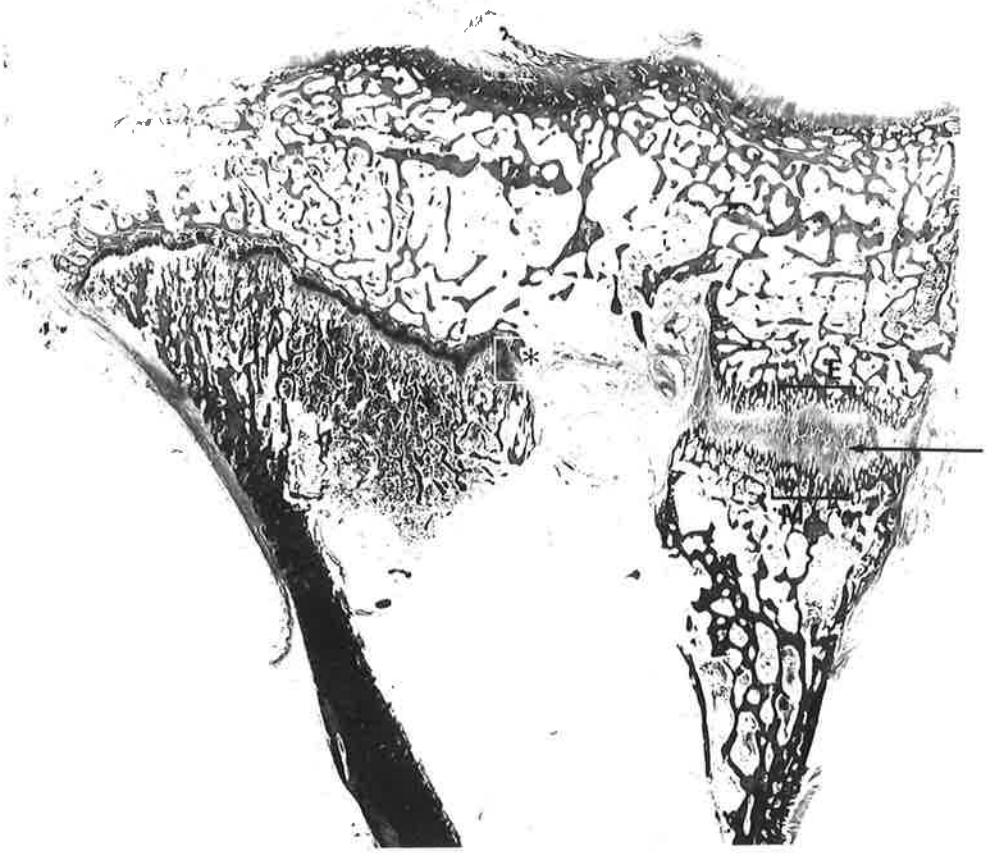
or

(ii) the remaining physis has sufficient potential to maintain growth.

In support of the last statement is the evidence that the larger the defects, the less likely is the possibility of reversal. The C.T. scans show a statistically significant increase in the defect size from group 1 to group 3 ($p < 0.0001$).

The chance of reversal in the 1 sq. cm. defect group is 100% if the interpositional material survives. 12 of 12 animals show improvement of radiological values. In the 2 sq. cm. group, where there was complete or incomplete necrosis in three animals, reversal occurred in only 6 of 11 animals. In the 3 sq. cm. group, where 100% of the fat survived, the reversal of deformity occurred only in 4 of 8 cases and the varus angulation improvement occurred in only 2 of those cases. There was no reversal of the angular deformity as great as noted in the 1 sq. cm. series.

Statistically the C.T. data proves a significant difference between group one and group three. i.e. between 17.2% $\pm$ 2.1% and 33.6% $\pm$ 6.7%, ($p < 0.0001$) and between group one and group two i.e. 17.2% $\pm$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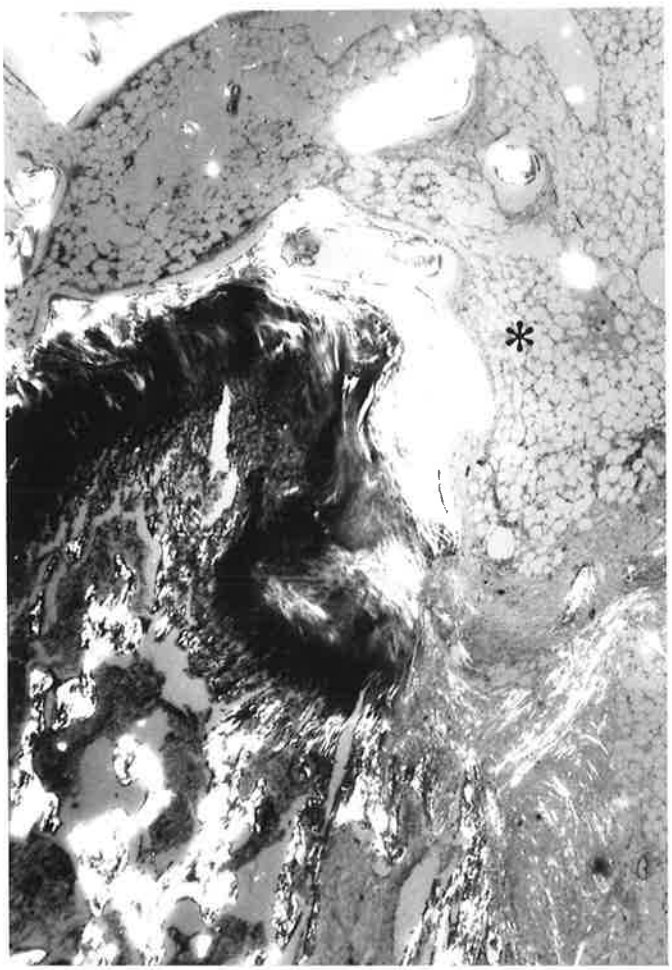
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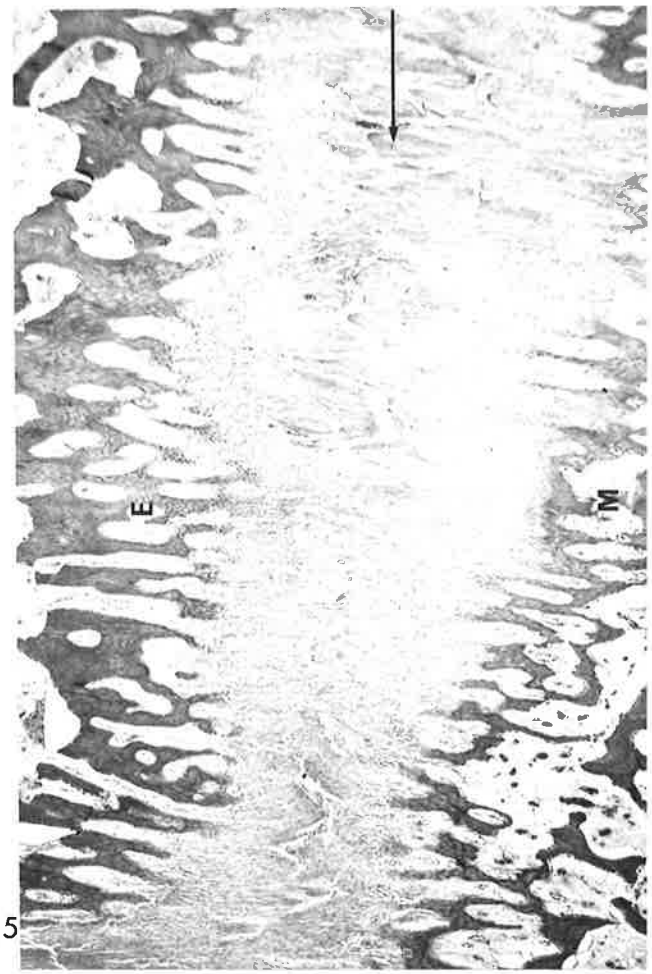
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2.1% and 28.0% ± 6.0% ($p < 0.0001$). This suggests that, where the defect is < 28% of the plate, if a stable environment for the interposition can be maintained, there will be resumption of longitudinal growth and correction of deformity. Larger defects may enable resumption of growth without correction of angulation. Whether at skeletal maturity a premature cessation of physeal activity occurs has not been examined.

It is proven that correction of length and angulation will occur in 100% of cases for the defect of 17.2%.

The C.T. scan data suggested that if a bone bridge can be prevented then a non-union effect will enable normal growth to proceed. If the initial size of the defect is too big the remnant growth plate cannot maintain growth sufficiently to prevent progression of a deformity.

Bright (1982) reported on a series of experiments on 130 puppies using Medical Elastomer #382 (X7 - 2320). He attempted to define the percentage of the physeal area that may be resected yet maintain longitudinal growth. He confirmed that the lateral distal femoral growth mechanism was more sensitive than the medial and that central defects would re-bridge if there was loss of contact of the interposition with the epiphysis. The mechanism for the resumption of longitudinal growth was not mentioned.

In attempting to document a decrease in size of the physeal defect the author's study did <u>not</u> quantitate that a decrease in size of the growth plate defect occurred with time, as has been proposed by Langenskiöld (1986), Langenskiöld, Österman & Valle (1987).

The C.T. at the time of reversal did show a change in the size of the defects (Tables 5C, 5H). In the 1 sq. cm. series 17.5 ± 2.0%

increased to 21.3 ± 4.4% at three months, 16.9 ± 2.5% decreased to 15.1 ± 4.6 at six months. In the 2 sq. cm. series 26.3 ± 5.3% decreased to 25.0 ± 7.3% at three months and 30.8 ± 6.9% decreased to 22.4 ± 3.7% at six months. In the 3 sq. cm. series 34.2% ± 8.4% increased to 34.7 ± 8.1% at three months and 32.7 ± 6.3% increased to 36.2 ± 9.6% at six months. Using the unpaired Student's *t* test there was not a statistically significant alteration in size between groups. However, if the defect does decrease in size it would seem more consistent that it is a result of relative diminution of the defect size compared to an increase in total physeal diameter with diametric growth rather than an interstitial repair process.

The longitudinal size of the defect was noted to be increased in 6 of 7 1 sq. cm. defects by six months. This noticeable increase in size of the defect is probably due to the growth potential in this group. The fat survives and maintains a position close to the epiphysis and as longitudinal growth occurs the lipocytes may proliferate to maintain a space within the stabilizing bone cavity. In other experiments it was noted that replacement of cultured chondrocyte implants occurred with haemopoeitic bone marrow fat by six months [Foster 1988]. The stable bone defect allows growth to proceed if the critical area of physeal resection is not exceeded.

Chapter 7

7.1 CONCLUSIONS:

1. Interpositional physiolysis with a free fat graft is confirmed as an appropriate strategy for prevention of deformity following peripheral bone bridge resection. It is suggested that, providing the defect is less than 17.2% (± 2.1%) of the total physeal area, success can be achieved in 100% of cases.

2. Fat as an interpositional material has the advantage of availability and biocompatibility but the disadvantage of:

   (i) potential loss of position due to either failure of haemostasis or loss of attachment to the epiphysis/physeal junction,

   (ii) fat necrosis with bone bridge reformation.

3. Results of Computerized Tomographic scanning have not shown a statistically significant diminution in the size of defect from three to six months. This questions the ability of the physis to repair with normal cartilage. The mechanism of the success of these interpositional procedures may relate to the ability of the physis to maintain its function against a physiological "non-union" produced by the interpositional material at the physeal level.

4. The repair of the central physeal cartilage is with phenotypically different fibrocartilage rather than specialized hyaline cartilage. This is characterized by metaplasia and irregularity of clones of chondrocytes.

5. Failure of interpositional procedures leads to bone bridge reformation across the physis. Histologically, the bone bridges may be cortical or cancellous, and may be located either centrally or peripherally, or both.

6. Provision of intact functioning physeal cartilage grafts may enable larger defects to be replaced with biologically active interpositional materials.

7.2 FUTURE DIRECTIONS:

Recent experimental investigations suggest that successful repair of articular cartilage defects may be possible [McKibbin 1971, Mitchell & Shepard 1976, Radin, Ehrlich & Chernack et al 1978, McKibbin & Ráliš 1978, Speer 1979, Salter 1980, 1982, Mankin 1982, O'Driscoll & Salter 1984, Miller, Ráliš & McKibbin 1985, O'Driscoll et al 1986, Zarnett 1987].

The requirements for such regeneration of articular cartilage include:

(i) a population of cells that can be placed into or will migrate into a cartilage defect, where they will proliferate and differentiate into chondrocytes,

(ii) a synthetic or biological matrix into which cells can penetrate and eventually replace or remodel into a cartilage framework; or cells capable of synthesizing such a matrix,

(iii) mechanical stimuli to enhance the cell proliferation and differentiation of chondrocytes,

(iv) protection of the repair cartilage from excessive loads,

and

(v) maintenance or restoration of the normal shape and conformation of the cartilage.

These criteria are similar for physeal cartilage regeneration.

Bentley (1978) has shown that physeal chondrocytes in culture can maintain phenotype for six weeks. Transplantation of these cells to articular defects may repair articular surface defects for up to one year [Aston & Bentley 1986]. Itay in 1987 successfully used cultured embryonic chick epiphyseal chondrocytes as grafts for defects in chick articular cartilage.

Trippel (1980), Amadio & Ehrlich (1983), have reported a high density culture technique and subsequent transfer to the physis of physeal chondrocytes [Kawabe, Ehrlich & Mankin 1987]. Despite culture techniques, and the immunological privilege of physeal cartilage all future experiments with allograft material will require histocompatibility barriers to be overcome. Additionally, the difficulty of maintaining phenotypical expression will need to be considered.

Other factors that stimulate chondrocyte repair may also have the potential to initiate cartilage differentiation. The mechanism of action may be by stimulating the mesenchymeal cell differentiation. Syftestad and Caplan (1986), report promising results with a 31-kD bone matrix protein. Another such agent may be the newly isolated osteogenin. This protein, which has an apparent molecular weight of 22-kD, in conjunction with insoluble collagenous matrix initiates cartilage formation [Sampath 1987].

Other approaches include the transforming growth factor beta, [Sporn 1986, Seyedin 1986] a 25-kD multifunctional growth regulator that induces cartilage proteoglycan synthesis in the mesenchymal cells of rat muscle [Cheung 1978]. Platelet-derived growth factor [Huang 1982], fibroblast growth factor [Zapf 1986], and insulin-like growth factor [Hauschka 1986] may all be potentially important in promoting physeal cartilage repair. The demonstration of site-specific somatomedin receptors on physeal chondrocytes may enable the stimulation of skeletal growth [Trippel 1988].

To provide a biologically active interpositional material experiments with physeal chondrocyte culture and allograft transfer are proceeding [Foster 1987, 1988, Hansen 1989 (in press)].

Appendix 1.

Sheep No.	Initial x-rays	operation side	control side	x-ray at 3 months	operation side	control side
1	Pin Distance Pin Angle Bone Length Bone Angle	23 mm 0 171 mm 4° valgus	24 mm 5° valgus 169 mm 1° valgus	Pin Distance Pin Angle Bone Length Bone Angle	27 mm 14° varus 194 mm 6° varus	39.5 mm 10°valgus 197 mm 8° valgus
2	Pin Distance Pin Angle Bone Length Bone Angle	23 mm 3.5° valgus 174 mm 2° valgus	25 mm 1° valgus 173.5 mm 3° valgus	Pin Distance Pin Angle Bone Length Bone Angle	42 mm 9° valgus 209.5 mm 4° valgus	42 mm 2° varus 207 mm 5°valgus
3	Pin Distance Pin Angle Bone Length Bone Angle	23 mm 3° valgus 166 mm 1° valgus	24 mm 3° valgus 172 mm 5° valgus	Pin Distance Pin Angle Bone Length Bone Angle	39 mm 5° valgus 196 mm 0.5°valgus	40 mm 4°valgus 201 mm 5° valgus
4	Pin Distance Pin Angle Bone Length Bone Angle	22 mm 3° valgus 158 mm 0	22.5 mm 2° varus 163 mm 0	Pin Distance Pin Angle Bone Length Bone Angle	38 mm 2° valgus 198 mm 4° varus	47 mm 9° valgus 215 mm 8° valgus
5	Pin Distance Pin Angle Bone Length Bone Angle	23 mm 4° valgus 172 mm 2° valgus	23 mm 6° valgus 172 mm 2° valgus	Pin Distance Pin Angle Bone Length Bone Angle	25.5 mm 11° varus 187.5 mm 19° varus	39.5 mm 4° valgus 208 mm 3° valgus
6	Pin Distance Pin Angle Bone Length Bone Angle	24 mm 6° valgus 171 mm 4° valgus	24 mm 1° valgus 169 mm 0	Pin Distance Pin Angle Bone Length Bone Angle	39 mm 3° varus 202 mm 2° valgus	45 mm 13°valgus 206 mm 3° valgus
7	Pin Distance Pin Angle Bone Length Bone Angle	24 mm 5° valgus 166 mm 4° valgus	24 mm 5° valgus 161.5 mm 5° valgus	Pin Distance Pin Angle Bone Length Bone Angle	21 mm 17° varus 186 mm 15° varus	43 mm 5° valgus 202.5 mm 7° valgus
8	Pin Distance Pin Angle Bone Length Bone Angle	23.5 mm 3.5° valgus 169 mm 3° valgus	23.5 mm 5.5°valgus 167.5 mm 1° valgus	Pin Distance Pin Angle Bone Length Bone Angle	39 mm 3° valgus 199.5 mm 3°valgus	42 mm 12°valgus 200 mm 2° valgus
9	Pin Distance Pin Angle Bone Length Bone Angle	24 mm 3° valgus 167 mm 1° valgus	25 mm 0 167 mm 1° valgus	Pin Distance Pin Angle Bone Length Bone Angle	23 mm 12° varus 195 mm 12° varus	40 mm 1°valgus 199 mm 4° valgus
10	Pin Distance Pin Angle Bone Length Bone Angle	24 mm 0 164 mm 6.5° valgus	24 mm 5° valgus 163 mm 5° valgus	Pin Distance Pin Angle Bone Length Bone Angle	22 mm 18° varus 181 mm 13° varus	40 mm 9°valgus 193 mm 5°valgus

Appendix 2

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side
11	Pin Distance	21 mm	24 mm	17 mm	41 mm	31 mm	43 mm
	Pin Angle	0	0	24° varus	9°valgus	12° valgus	8° valgus
	Bone Length	154 mm	155 mm	179 mm	189 mm	180 mm	188 mm
	Bone Angle	1° valgus	2° valgus	19.5° varus	4° valgus	10° varus	0
Pin repositioned	Pin Distance			24 mm	39 mm		
	Pin Angle			3° valgus	5° valgus		
	Bone Length			170 mm	180 mm		
	Bone Angle			15° varus	2° valgus		
12	Pin Distance	23 mm	23 mm	22 mm	39 mm	34 mm	44 mm
	Pin Angle	2° varus	2° valgus	12° varus	10° valgus	6° varus	9° valgus
	Bone Length	155 mm	155 mm	177 mm	184 mm	187 mm	193 mm
	Bone Angle	1° varus	1° varus	15° varus	6° valgus	4° varus	6° valgus
13	Pin Distance	21 mm	23 mm	21 mm	35 mm	33 mm	46 mm
	Pin Angle	3° varus	2° valgus	17° varus	8° valgus	13° varus	7° valgus
	Bone Length	142 mm	416 mm	158 mm	167 mm	176 mm	188 mm
	Bone Angle	2° varus	1° varus	14° varus	5° valgus	3° varus	5° valgus
14	Pin Distance	24 mm	23 mm	22 mm	34 mm	34 mm	41.5 mm
	Pin Angle	3° valgus	2° valgus	15° varus	3° valgus	4° varus	0
	Bone Length	155 mm	157 mm	168 mm	177 mm	184 mm	196 mm
	Bone Angle	1° varus	1° valgus	13° varus	1° valgus	7° varus	1° varus
15	Pin Distance	23.5 mm	23.5 mm	23 mm	39.5 mm	24 mm	45 mm
	Pin Angle	0	3° valgus	18° varus	5° valgus	21° varus	4.5° valgus
	Bone Length	158 mm	159 mm	179 mm	189 mm	187.5 mm	195.5 mm
	Bone Angle	4° valgus	5° valgus	17° varus	1° valgus	22° varus	2° valgus
16	Pin Distance	23 mm	23 mm	21 mm	39 mm	23 mm	45 mm
	Pin Angle	2° valgus	5° valgus	19° varus	4° valgus	22° varus	5° valgus
	Bone Length	155 mm	154 mm	180 mm	184 mm	188 mm	195 mm
	Bone Angle	1° valgus	5° valgus	18° varus	5° valgus	18° varus	3° valgus
17	Pin Distance	21 mm	22 mm	20 mm	42 mm	31 mm	51 mm
	Pin Angle	1° valgus	1° valgus	22° varus	6° valgus	17° varus	6° valgus
	Bone Length	156 mm	164 mm	193 mm	202 mm	209 mm	218 mm
	Bone Angle	0	3° valgus	23° varus	3° valgus	15° varus	3° valgus

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)		x-ray at 9 months (6 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side	operation side	control side
18	Pin Distance	23 mm	23 mm	21 mm	36 mm	38 mm	51 mm	51 mm	64 mm
	Pin Angle	1° valgus	2° valgus	20° varus	4° valgus	7° varus	7° valgus	4° varus	4° valgus
	Bone Length	156 mm	155 mm	171 mm	179 mm	206 mm	211 mm	216 mm	225 mm
	Bone Angle	0	0	16° varus	5° valgus	1° varus	1° varus	1° valgus	2.5° valgus
19	Pin Distance	22 mm	22 mm	21 mm	31 mm	31 mm	38 mm	40 mm	48 mm
	Pin Angle	0	2° valgus	17° varus	6° valgus	11° varus	4° valgus	11° varus	1° valgus
	Bone Length	138 mm	137 mm	152 mm	156 mm	163 mm	170 mm	182 mm	184 mm
	Bone Angle	2° valgus	0	12° varus	3° valgus	1° valgus	2° varus	5° varus	2° varus
20	Pin Distance	22 mm	22 mm	22 mm	28 mm	34 mm	36 mm	47 mm	51 mm
	Pin Angle	0	2° valgus	10° varus	5° valgus	3° varus	3° valgus	3° varus	0
	Bone Length	144 mm	147 mm	154 mm	158 mm	165 mm	173 mm	200 mm	204 mm
	Bone Angle	2° valgus	1° valgus	12° varus	2° valgus	3° varus	0	3° varus	1° varus
21	Pin Distance	22 mm	22 mm	22 mm	31 mm	34 mm	37 mm	47 mm	50.5 mm
	Pin Angle	3° valgus	0	11° varus	1° valgus	2° varus	1° valgus	0	1° varus
	Bone Length	148 mm	148 mm	156 mm	160 mm	172 mm	173 mm	203 mm	205 mm
	Bone Angle	0	1° varus	9° varus	2° valgus	1.5° varus	2° varus	2° varus	1° valgus
22	Pin Distance	22 mm	23 mm	22 mm	36 mm	33 mm	43 mm	46 mm	55.5 mm
	Pin Angle	0	0	18° varus	1° valgus	8° varus	0	7° varus	0
	Bone Length	140 mm	140 mm	156 mm	162 mm	166 mm	169 mm	197 mm	199.5 mm
	Bone Angle	2° varus	1° varus	14° varus	4° valgus	6.5° varus	3° valgus	6° varus	1° valgus
23	Pin Distance	22.5 mm	19 mm	23 mm	36.5 mm	31.5 mm	41.5 mm	38 mm	47 mm
	Pin Angle	0	4° varus	15° varus	8° valgus	7° varus	9° valgus	7° varus	8° valgus
	Bone Length	159 mm	159 mm	186 mm	194.5 mm	199 mm	202 mm	207.5 mm	216 mm
	Bone Angle	1° valgus	1° valgus	12° varus	5.5°valgus	4° varus	6° valgus	5° varus	1° valgus
24	Pin Distance	22 mm	22.5 mm	21 mm	36 mm	31.5 mm	42 mm	39 mm	49.5 mm
	Pin Angle	2° varus	0	18° varus	9° valgus	10° varus	7° valgus	8° varus	6° valgus
	Bone Length	154.4 mm	157 mm	178.5 mm	187 mm	182 mm	192 mm	205 mm	206 mm
	Bone Angle	0	0.5° valgus	14° varus	2° valgus	7° varus	0	5° varus	5° valgus

Appendix 3

1 SQ. CM. C.T. RESULTS

3 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
11	Initial	$54.2/9.25$	17.1	$53.75/9.24$	17.2	$54.07/9.25$	17.1	17.1
	Final	$69.1/15.7$	22.7	$61.9/17.3$	27.9	$62.5/18.0$	28.8	26.5
12	Initial	$60.09/12.26$	17.7	$70.4/12.33$	17.5	$70.09/12.99$	18.5	17.9
	Final	$68.8/14.8$	21.5	$64.2/13.8$	21.5	$64.3/13.9$	21.6	21.5
13	Initial	$68.21/11.27$	16.5	$67.11/10.76$	16.03	$67.81/11.74$	17.3	16.6
	Final	$65.6/11.6$	17.7	$62.3/12.5$	20.1	$61.4/11.0$	17.9	18.6
14	Initial	$69.53/10.56$	15.2	$69.05/10.71$	15.5	$68.33/11.01$	16.1	15.6
	Final	$56.5/12.4$	22.0	$55.2/13.2$	23.9	$58.7/12.5$	21.3	22.4
15	Initial	$84.6/15.3$	18.1	$84.2/15.3$	18.2	$85.0/15.8$	18.6	18.3
	Final	$77.1/14.9$	19.3	$76.1/16.4$	21.6	$74.6/15.6$	20.9	20.6
16	Initial	$91.7/19.4$	21.2	$91.6/19.2$	21.0	$91.0/19.5$	21.4	21.2
	Final	$100.3/25.5$	25.4	$98.4/25.7$	26.1	$96.2/25.4$	26.4	26.0
17	Initial	$107.0/16.3$	15.2	$107.7/17.2$	16.0	$107.0/16.3$	15.2	15.5
	Final	$115.1/14.4$	12.5	$116.2/16.2$	13.9	$112.1/16.2$	14.5	13.6

1 SQ. CM. C.T. RESULTS

6 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
18	Initial	$66.08/11.21$	17.0	$65.10/10.76$	16.5	$63.45/10.18$	16.0	16.5
	Final	$80.3/13.8$	17.2	$76.7/14.0$	18.3	$75.5/13.3$	17.6	17.7
19	Initial	$50.05/10.5$	21.0	$48.95/9.94$	20.3	$50.39/10.75$	21.3	20.9
	Final	$52.6/9.9$	18.8	$51.5/9.0$	17.5	$49.7/9.3$	18.7	18.3
20	Initial	$52.77/9.0$	17.1	$53.18/9.5$	17.9	$53.24/9.25$	17.4	17.5
	Final	$60.2/9.9$	16.5	$59.4/9.9$	16.7	$60.6/10.3$	17.0	16.7
21	Initial	$55.35/10.32$	18.6	$56.0/11.17$	20.0	$53.35/9.55$	17.9	18.8
	Final	$64.4/12.3$	19.1	$68.2/13.5$	19.8	$66.4/12.8$	19.3	19.4
22	Initial	$62.85/11.16$	17.8	$62.52/10.11$	16.2	$61.70/10.74$	17.4	17.1
	Final	$80.9/12.8$	15.8	$80.8/11.7$	14.5	$78.4/13.0$	16.6	15.6
23	Initial	$79.7/9.3$	11.7	$79.7/11.0$	13.8	$80.0/12.1$	15.1	13.5
	Final	$2.92/43.0$	6.8	$2.8/43.3$	6.5	$2.5/42.4$	5.9	6.4
24	Initial	$70.2/10.7$	15.2	$68.6/9.4$	13.7	$71.6/9.9$	13.8	14.2
	Final	$6.3/55.8$	11.4	$6.3/55.2$	11.5	$6.6/53.8$	12.3	11.7

Appendix 4

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side
25	Pin Distance	22 mm	24 mm	22 mm	37 mm	28 mm	45 mm
	Pin Angle	0	3° valgus	18° varus	3° valgus	11° varus	5° valgus
	Bone Length	134 mm	135 mm	156 mm	155 mm	162 mm	176 mm
	Bone Angle	5° varus	0.5° varus	10° varus	3° valgus	13° varus	3° varus
26	Pin Distance	21 mm	22 mm	20 mm	32.5 mm	31 mm	44 mm
	Pin Angle	4° valgus	2° valgus	13° varus	5° valgus	13° varus	6° valgus
	Bone Length	128 mm	129 mm	139 mm	147.5 mm	165 mm	169 mm
	Bone Angle	2° varus	3° varus	19° varus	5° valgus	15° varus	5° valgus
27	Pin Distance	23 mm	22 mm	24 mm	34 mm	29 mm	50 mm
	Pin Angle	2° valgus	7° varus	15° varus	6° varus	20° varus	9° valgus
	Bone Length	145 mm	142 mm	160 mm	170 mm	180 mm	184 mm
	Bone Angle	6° varus	12° varus	17° varus	1° valgus	20° varus	4° valgus
28	Pin Distance	22 mm	22 mm	22 mm	38 mm	28.5 mm	50.5 mm
	Pin Angle	3° valgus	5° valgus	23° varus	7° valgus	10° varus	8° valgus
	Bone Length	136 mm	135 mm	161 mm	167 mm	183 mm	187 mm
	Bone Angle	1° valgus	6° varus	22.5° varus	2° valgus	20° varus	0
29	Pin Distance	22 mm	21 mm	16 mm	30.5 mm	20 mm	42 mm
	Pin Angle	0	0	38° varus	1° varus	40° varus	1° varus
	Bone Length	133 mm	132 mm	151 mm	151 mm	174 mm	179 mm
	Bone Angle	1° varus	1° varus	26° varus	5° varus	18.5° varus	0
30	Pin Distance	22.5 mm	23.5 mm	22 mm	36 mm	25 mm	46 mm
	Pin Angle	7° valgus	6° valgus	9° varus	8° valgus	18.5° varus	10° valgus
	Bone Length	146.5 mm	146 mm	168 mm	171 mm	188 mm	192 mm
	Bone Angle	4° valgus	9° valgus	17° varus	1° valgus	14° varus	5° valgus
31	Pin Distance	22 mm	23 mm	23 mm	39 mm	28 mm	42 mm
	Pin Angle	2° valgus	1° valgus	15° varus	1° valgus	12° varus	1° varus
	Bone Length	165 mm	163 mm	194 mm	205 mm	214 mm	214 mm
	Bone Angle	4° valgus	2° valgus	19° varus	1° valgus	15° varus	7° varus

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)		x-ray at 9 months (6 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side	operation side	control side
32	Pin Distance	22.5 mm	23.5 mm	21 mm	34 mm	32 mm	45.5 mm	37.5 mm	50.5 mm
	Pin Angle	2° valgus	1° valgus	15° varus	0	8° varus	4° valgus	6.5° varus	3° valgus
	Bone Length	156 mm	157 mm	169 mm	176 mm	189 mm	195 mm	200 mm	207 mm
	Bone Angle	14 valgus	1° valgus	14° varus	2° valgus	12° varus	1° valgus	4° varus	7° valgus
33	Pin Distance	22 mm	23 mm	32 mm	38 mm	40 mm	47.5 mm	50 mm	59.5 mm
	Pin Angle	2° valgus	0	4° varus	5° valgus	2° varus	5° valgus	1° varus	3° valgus
	Bone Length	151 mm	151 mm	176 mm	179 mm	195 mm	195 mm	213 mm	218 mm
	Bone Angle	3° varus	2.5° varus	5° varus	2° varus	2° varus	3.5° valgus	0	1.5° valgus
34	Pin Distance	23 mm	23 mm	21 mm	36 mm	33 mm	49 mm	41 mm	60 mm
	Pin Angle	6° valgus	2° valgus	17° varus	4° valgus	18° varus	4° valgus	21° varus	3° varus
	Bone Length	152 mm	152 mm	168 mm	178 mm	188 mm	199.5 mm	210 mm	220 mm
	Bone Angle	0	4° valgus	18° varus	2° valgus	15° varus	5° valgus	17° varus	2° valgus
35	Pin Distance	22 mm	22 mm	20 mm	35 mm	36 mm	49.5 mm	37 mm	64 mm
	Pin Angle	4° valgus	1° valgus	17° varus	5° valgus	10° varus	4° varus	20° varus	9° valgus
	Bone Length	137 mm	137 mm	151 mm	156 mm	192 mm	197 mm	204 mm	212.5 mm
	Bone Angle	3° varus	0	20° varus	4° valgus	10° varus	1° valgus	22.5° varus	5° valgus

Appendix 5

2 SQ. CM. C.T. RESULTS

3 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
25	Initial	63.3/19.3	30.5	63.3/19.1	30.1	64.5/20.1	31.2	30.6
	Final	67.9/20.7	30.5	67.9/22.3	32.8	67.8/19.3	28.5	30.6
26	Initial	58.5/12.8	21.8	57.6/12.4	21.5	57.8/12.7	22.0	21.8
	Final	61.9/14.8	24.0	59.2/14.3	24.2	58.0/13.7	23.6	23.9
27	Initial	50.3/14.4	28.6	49.7/14.4	29.1	49.4/14.9	30.2	29.3
	Final	83.7/20.7	24.7	86.6/21.0	24.3	82.5/19.9	24.1	24.4
28	Initial	74.2/21.7	29.2	75.3/23.1	30.6	74.3/22.9	30.8	30.2
	Final	73.9/28.9	39.1	73.0/26.1	35.8	68.7/24.6	35.8	36.9
29	Initial	70.3/12.7	18.1	69.4/13.4	19.3	68.5/11.5	16.8	18.1
	Final	70.2/16.8	23.9	65.8/17.0	25.8	72.9/15.2	20.9	23.5
30	Initial	83.2/25.7	30.9	82.0/25.8	31.5	82.2/25.8	31.4	31.3
	Final	7.7/32.1	24.1	7.3/33.6	21.7	7.6/33.2	22.9	22.9
31	Initial	112.9/27.0	23.9	110.0/25.3	23.0	110.7/24.9	22.5	23.1
	Final	9.0/66.6	13.5	9.0/68.6	13.1	8.9/68.4	13.4	13.3

6 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
32	Initial	64.78/19.41	30.0	63.6/19.3	30.4	61.3/17.7	28.9	29.8
	Final	9.5/45.4	21.0	9.9/43.7	22.7	9.1/45.2	20.2	21.3
33	Initial	62.1/14.0	22.6	59.77/13.5	22.6	59.8/14.3	23.8	23.0
	Final	8.8/46.08	19.0	8.9/44.6	20.0	8.3/45.8	18.2	19.1
34	Initial	52.5/15.7	30.0	52.6/17.1	32.5	51.6/15.2	29.5	30.7
	Final	9.9/44.1	22.5	9.3/44.6	20.8	9.7/45.2	21.4	21.6
35	Initial	58.5/22.8	39.0	57.3/23.3	40.6	57.7/23.0	39.9	39.8
	Final	12.1/40.1	30.5	11.0/40.1	27.5	10.2/40.3	25.2	27.7

Appendix 6

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side
36	Pin Distance	22 mm	23 mm	17 mm	39 mm	14.5 mm	53.5 mm
	Pin Angle	0	3° valgus	23° varus	8° valgus	33° varus	5° valgus
	Bone Length	155 mm	158 mm	178 mm	186.5 mm	192 mm	207 mm
	Bone Angle	0	2° valgus	19° varus	2° valgus	31° varus	4° valgus
37	Pin Distance	24.5 mm	21 mm	20.5 mm	38 mm	26 mm	46 mm
	Pin Angle	1° valgus	3° valgus	14° varus	5° valgus	14° varus	3.5° valgus
	Bone Length	129 mm	134 mm	150 mm	163.5 mm	169 mm	178 mm
	Bone Angle	4° valgus	4° valgus	16° varus	8° valgus	18° varus	8° valgus
38	Pin Distance	24 mm	23.5 mm	21 mm	41.5 mm	24.5 mm	45.5 mm
	Pin Angle	2° valgus	3° valgus	19° varus	6° valgus	17° varus	4.5° valgus
	Bone Length	167.5 mm	174 mm	203 mm	206.5 mm	210 mm	221 mm
	Bone Angle	5° valgus	0	15° varus	1° varus	16° varus	1° valgus
39	Pin Distance	21.5 mm	25 mm	21 mm	39.5 mm	25.5 mm	41.5 mm
	Pin Angle	1° valgus	5° valgus	11.5° varus	5° valgus	7° varus	6° valgus
	Bone Length	168.5 mm	170 mm	190 mm	194 mm	-	-
	Bone Angle	4° valgus	1° valgus	10.5° varus	4° valgus	12° varus	10° varus
40	Pin Distance	22 mm	21 mm	16 mm	37.5 mm	22 mm	41 mm
	Pin Angle	2° valgus	2° valgus	10.5° varus	1° valgus	5° varus	3° valgus
	Bone Length	181 mm	176 mm	204 mm	210.5 mm	215 mm	224 mm
	Bone Angle	0	5° valgus	13° varus	4° valgus	20° varus	3° varus

Sheep No.		Initial		x-ray at 3 months		x-ray at 6 months (3 months post reversal)		x-ray at 9 months (6 months post reversal)	
		operation side	control side	operation side	control side	operation side	control side	operation side	control side
41	Pin Distance	24 mm	24 mm	23.5 mm	39.5 mm	35 mm	51 mm	36.5 mm	52.5 mm
	Pin Angle	0	1° valgus	15° varus	6° valgus	11° varus	3° valgus	9° varus	3° valgus
	Bone Length	173 mm	173 mm	187 mm	198.5 mm	204 mm	210 mm	206 mm	217 mm
	Bone Angle	3° valgus	3° valgus	14° varus	0	10° varus	0	-	1° valgus
42	Pin Distance	24 mm	25.5 mm	23 mm	39 mm	38 mm	51 mm	40 mm	53 mm
	Pin Angle	1° valgus	1° valgus	7° varus	7° valgus	0	0	0	1° valgus
	Bone Length	164 mm	165 mm	179.5 mm	189 mm	198 mm	207 mm	206 mm	212 mm
	Bone Angle	5° varus	1° varus	11° varus	1° valgus	7° varus	0	3° varus	2° valgus
43	Pin Distance	22.5 mm	22 mm	21 mm	32.5 mm	21 mm	41 mm	26 mm	46.5 mm
	Pin Angle	0	3° varus	18° varus	9° valgus	21° varus	20° valgus	23° varus	26° valgus
	Bone Length	142.5 mm	143 mm	151 mm	158 mm	164 mm	178 mm	+ 75 mm	+ 88 mm
	Bone Angle	2° varus	2° varus	9.5° varus	1° varus	18° varus	2° valgus	11° varus	0

Appendix 7

3 SQ. CM. C.T. RESULTS

3 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
36	Initial	$79.9/28.7$	35.9	$83.5/30.3$	36.3	$83.2/29.2$	35.1	35.8
	Final	$10.6/42.2$	25.1	$10.2/41.2$	24.7	$10.4/43.1$	24.1	24.6
37	Initial	$68.6/34.0$	49.6	$68.3/32.2$	47.1	$69.3/31.9$	46.0	47.6
	Final	$18.4/48.8$	37.7	$19.0/50.2$	37.9	$18.8/49.6$	37.8	37.8
38	Initial	$92.8/21.5$	23.2	$93.6/25.2$	26.9	$90.2/22.9$	25.4	25.2
	Final	$8.8/53.0$	16.5	$8.4/52.8$	15.9	$8.0/52.9$	15.1	15.8
39	Initial	$115.1/14.4$	12.5	$116.2/16.2$	13.9	$112.1/16.2$	14.5	13.6
	Final	$12.4/49.3$	25.2	$11.3/48.7$	23.2	$11.7/49.7$	23.6	24.0
40	Initial	$111.8/34.9$	31.2	$112.5/35.2$	31.3	$112.2/33.9$	30.2	30.9
	Final	$68.8/14.8$	21.5	$64.2/13.8$	21.5	$64.3/13.9$	21.6	21.5

6 MONTH SURVIVAL

Animal No.	C.T.	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Total Area/ G.P. Area	G.P. %	Ave %
41	Initial	$99.1/27.2$	27.4	$100.4/26.8$	26.7	$99.3/27.7$	27.9	27.3
	Final	$19.5/62.0$	31.4	$18.1/61.6$	29.4	$18.1/61.9$	29.2	30.0
42	Initial	$88.3/28.0$	31.7	$89.4/27.2$	30.4	$88.6/28.0$	31.6	31.2
	Final	$19.4/61.5$	31.6	$20.1/60.6$	33.2	$18.3/62.2$	29.5	31.4
43	Initial	$73.0/31.2$	42.7	$74.3/28.2$	38.0	$73.8/28.4$	38.5	39.7
	Final	$25.1/51.4$	48.8	$25.2/53.9$	46.7	$25.8/55.9$	46.1	47.2

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