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HEALING IN THE EXPERIMENTALLY FRACTURED OS PRIAPI OF THE RAT

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Among the naturally occurring "extra-skeletal" bones found in various mammalian species, the os priapi occurs in many, is easily accessible for fracturing, and its elongated form lends itself for comparison with the long supporting bones of the skeleton proper. A search of the literature revealed no previous study of healing in an os priapi.

Healing of the shaft of the os priapi was considered in light of the observations and hypotheses developed from many studies of skeletal fractures and lesions. Firstly, the environment of dense connective tissue in which the bone lies might predispose to a fibrous union. Secondly, the bone develops partly in cartilage and partly by direct ossification. McLean & Urist (1961) at one time suggested that only bones formed endochondrally develop cartilage in their callus of fracture: an analysis of the callus constitution in fractures at various points along the length of the shaft might reveal whether their view is justified for this bone. Thirdly, the relative contribution of periosteum and endosteum to fracture callus is still debated. The shaft of the os priapi is long in form but has no marrow cavity, only narrow vascular channels. Can a fracture of it therefore be viewed as comparable with a skeletal lesion elsewhere involving only cortical bone; and how do its vascular channels react? Fourthly, it would be of interest to compare the healing of the os priapi with that reported for the long bones, skull, and mandible of the rat and other species in regard to the nature and speed of the repair, the width of gap which can be bridged, and the influence of age on the course of healing. With these considerations in mind, fractures of the os priapi were made in two groups of rats—young and mature—and their healing was followed histologically.

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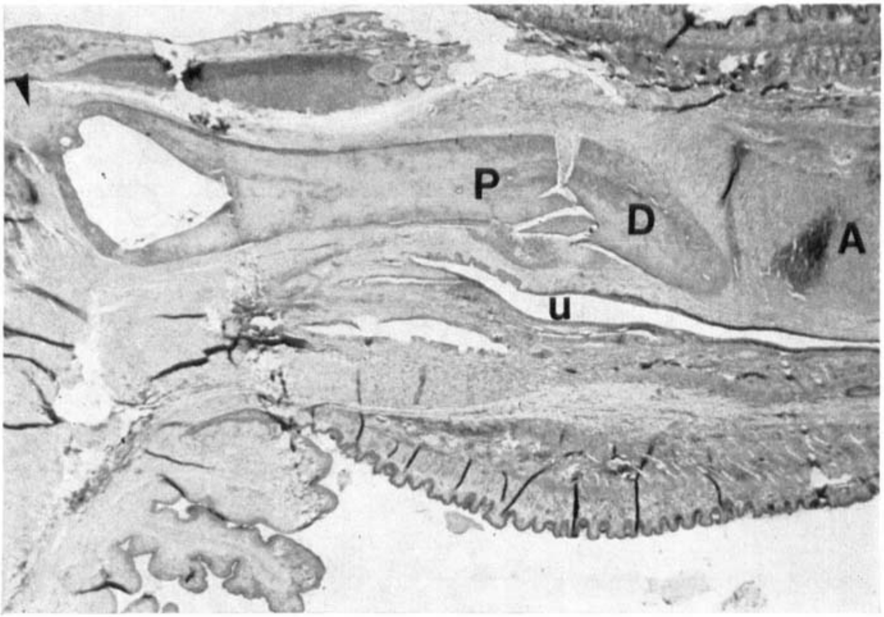

Figure 1. Four weeks after a manual fracture two-thirds along a mature shaft, union has been achieved by a ventral callus. The base of the bone's proximal enlargement has a dorsal spur of fibrocartilage (arrow). Endochondral growth at the base has ceased. The distal bone piece is attached to part of the "fibrous cap" or anterior process. × *17.*

Abbreviations: A = the anterior process; D = the distal bone piece after fracture; P = the proximal bone piece after fracture; u = the urethra.

MATERIALS AND METHODS

One hundred and four hooded Norwegian rats were used. The form and development of the rat's penile bone and its anterior process have been described by Ruth (1934) and Wiesner (1934). The bone in its full extent is shown in Figure 1. In nine, the attempts at fracture broke only the anterior process or else failed to interrupt the shaft. Nineteen successful fractures were made but became infected or the distal bone fragment was lost. Seventy-six rats were therefore available for study: they were grouped as follows.

Groups 1a and b. Group 1a comprised twenty-five young animals (between five and seven weeks of age) weighing between 96 and 182 g. Group 1b consisted of twenty-nine mature rats weighing between 340 and 665 g. Under ether anaesthesia, the penis was protruded through the prepuce, clamped across its base, and swabbed clean with 70 per cent ethyl alcohol. The shaft of the os priapi was cut through with pointed scissors via a dorsolateral approach. The soft tissue wound was closed with two or three interrupted sutures in the mature rats, but left open in the

Table 1. Severed fractures of os priapi
Group Ia, Mature

Survival period (weeks)	Fracture gap's width (mm) and kind of union 0 = non-union; Uc = union by cartilage; Ub = union by bone 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	>1.0
	0										
0– 2	0	0									
	0										
			0								
2– 5		Uc	0 Uc	0 Uc	Uc						0
			0	0							
5–10			Uc	Uc				Uc	0	Uc	
				Uc				Uc			
10–26		Ub		Uc Ub			0 Ub	0 Ub	0		

Total animals = 29; total Uc and Ub = 15

Group Ib, Young

0– 2	0	0	0	0						0	
		0									
2– 5	Uc				0 Uc	Uc	0	Uc			
						Uc					
5–10			0			0		0 Uc			
10–30		Ub									
		Ub		Ub	Ub		0				
		Ub		Ub	Ub						

Total animals = 25; total Uc and Ub = 13.

Table 2. Manually broken os priapi

Survival period (weeks)	Fracture gap's width (mm) and kind of union <0.1				0.1	0.2
2– 5	Uc	Uc	Uc		Uc	
5–10	Ub	Ub	Ub	Ub	Ub	Uc
10–12	Ub				Ub	

Total animals = 12; total Uc and Ub = 12.

younger ones. The clamp was removed and the penis pushed back, un-splinted, into the prepuce.

Group 2. In twelve, mature rats, weighing from 360–670 g, closed fractures of the shaft were made manually.

Group 3. Ten, unoperated rats of various ages served as controls indicating the state of development of the intact bone for comparison with the operated rats in Group 1a.

After a celibate survival of from four days to thirty weeks, the animals were killed with chloroform. The distal penis was fixed in Heidenhain's Suza solution before decalcification in a solution containing 5 per cent formic acid and 10 per cent formalin. The penis was imbedded in paraffin, cut longitudinally at 7 μ and the sections stained with haematoxylin and eosin or with van Gieson's stain.

The width of the fracture gap at its narrowest point, dead bone to dead bone, was measured during microscopy using a stage micrometer, and the results are given in Tables 1 and 2. To arrive at the factor by which the tissues had shrunk during processing, several penises were measured for length (ln) at the time of fixation and later as a section (ls) on the slide: ls/ln was 0.8.

RESULTS

General Features

The successful, sixty-six, fractured bones regularly had some separation of the resulting distal and proximal pieces; and, as an indication

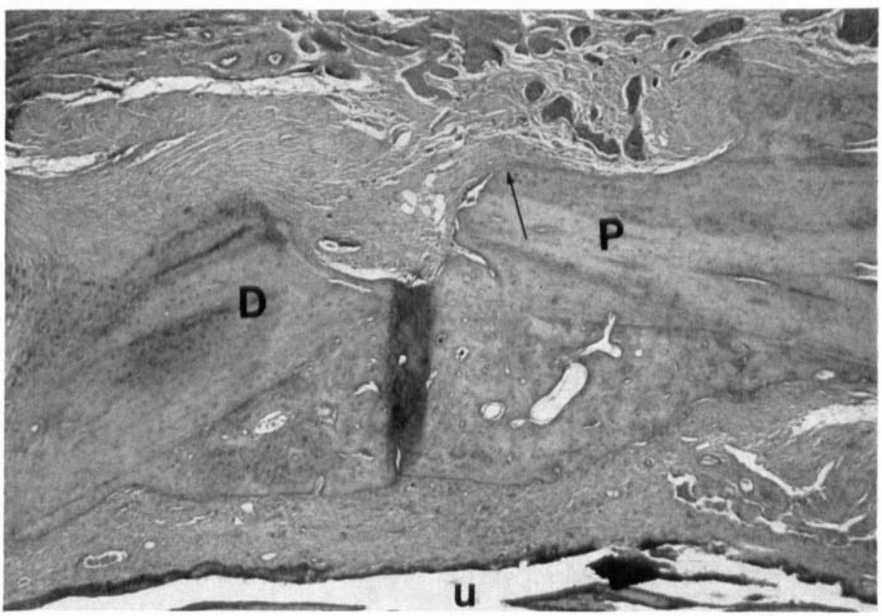

Figure 2. Sixteen weeks after severance of a mature bone, the angulated bone pieces are united by a dense bony ventral callus. Most of the gap is densely fibrous, and the dorsal corner of the proximal margin (arrow) has been rounded off. The dorsal callus lies some way back from that margin. The distal piece's end-face has a thin layer of new bone. × *42.*

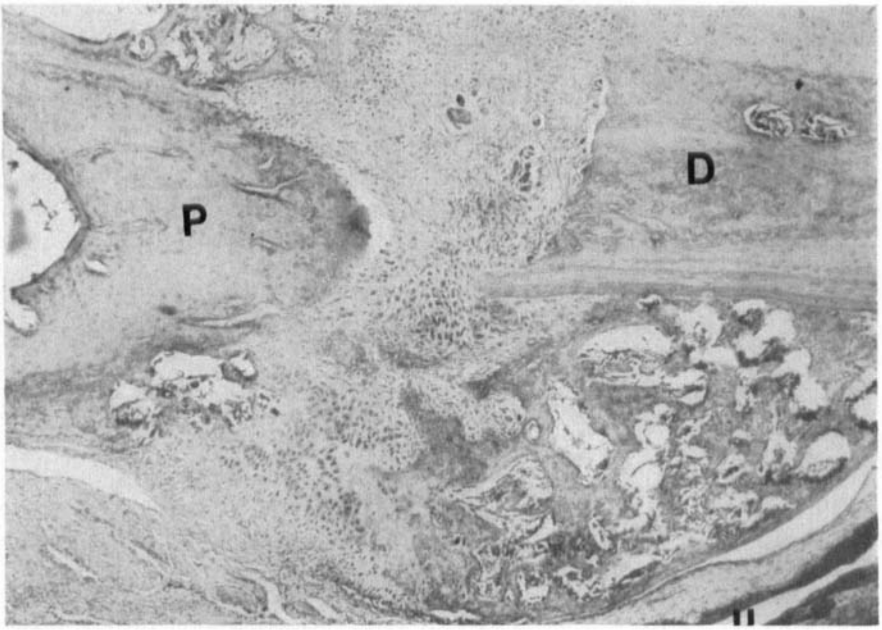

Figure 3. Three weeks after a manual fracture of a mature shaft, cartilaginous union has been achieved by a strong, ventral, periosteal outgrowth of callus. The distal piece is deficient dorsally in callus, thus no true periosteal collar has developed for this piece. Dense fibrous tissue separates the urethral epithelium from the ventral callus. × 67.

of the instability of their gap, the pieces were angulated, once to 80° of misalignment but usually to 10–40° (Figure 2). Bone fragments occasionally separated from the shaft ends, but comminution was never a marked feature. The instability was arrested in some animals by the achievement of bony or cartilaginous bridging of the gap. The gap width (uncorrected for shrinkage), kind of union, and approximate survival period for each animal with a severed os are presented in Table 1, and for those with a manually broken shaft in Table 2. Each animal is represented by letters: Ub denotes a continuous link of new bone across the gap; Uc that the osseous link was interrupted by cartilage; and 0 that bridging by any kind of cartilage or bone had not taken place.

Cartilaginous union sometimes took place as early as two weeks after severance, but six animals survived for several weeks with gaps as narrow as 0.4 mm (corrected approximately by the factor for shrink-

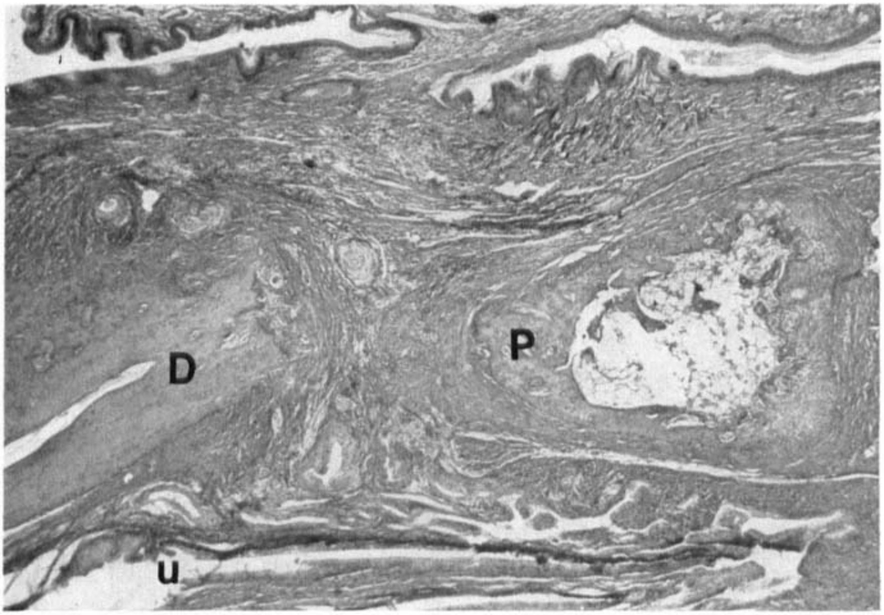

Figure 4. Three weeks after severance in a young rat, the fracture gap is fibrous and the bone pieces angulated. Active endochondral growth is present along the base of the proximal enlargement with its marrow cavity. A vascular channel runs within the distal bone. × *42.*

age) remaining fibrous. The frequency of union, in animals living post-operatively for two weeks or more, is not significantly ($\chi^2 = 0.35$) higher in the younger group of rats: 13/19 compared with 15/25 for the mature rats.

The separation was much less in the animals with manually broken shafts (Figure 1) (Table 2); union was seen in every animal; and the first bony bridging was noted at five weeks instead of ten weeks required for a cut os.

Events in the Fracture Gap and at the Bone-Ends

The angulation of the shaft pieces resulted in a fracture gap which was narrow on the ventral, urethral side, but wider on the dorsal side of the os priapi. The gap was bounded by the two bone margins and, in dorsal and ventral areas, by severed dense fibrous tissue, with the urethra close by ventrally. Young fibroblastic tissue grew into the gap

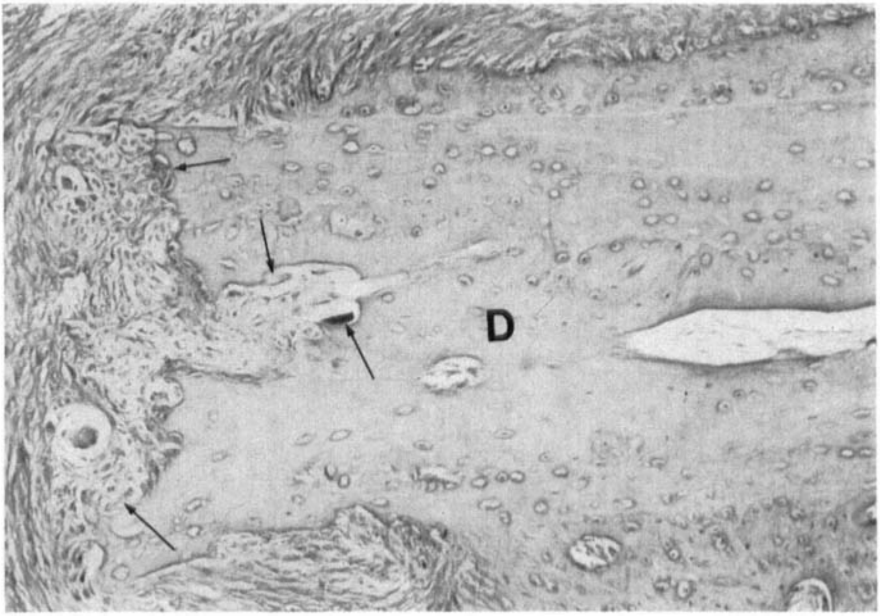

Figure 5. Three weeks after severance of a young bone, distal end-face and open vascular canal have several sites of osteoclastic erosion (arrow). There is a slight onlay of new bone just within the eroded canal. Young fibroblastic tissue occupies the gap. × 67.

(Figures 3 and 5) and, in the instances of persistent non-union, could become dense (Figure 4) and oriented transversely across the gap.

The distribution of the callus growing from the shaft ends bore a consistent relation to the angulation (Figures 1 and 2). The dorsal sides of the shaft usually developed only a little callus and often this was situated some way back from the cut margin, because the dorsal corner was undergoing erosion to a rounded, smoother contour (Figure 2). The ventral shaft surfaces developed considerably more callus tissue. Hyaline cartilage formed near the margin, and new bone grew under the periosteum further back from the edge and advanced into the cartilage by endochondral ossification (Figure 3). Some of this callus cartilage extended to cover a little of the cut end-face, but most of the length of the cut margin was in contact with other kinds of tissue to be described later.

In some animals, the ventral calluses of hyaline cartilage and new bone grew out sufficiently in the first two or three weeks to meet and fuse, thereby achieving an early cartilaginous union. Seldom, however,

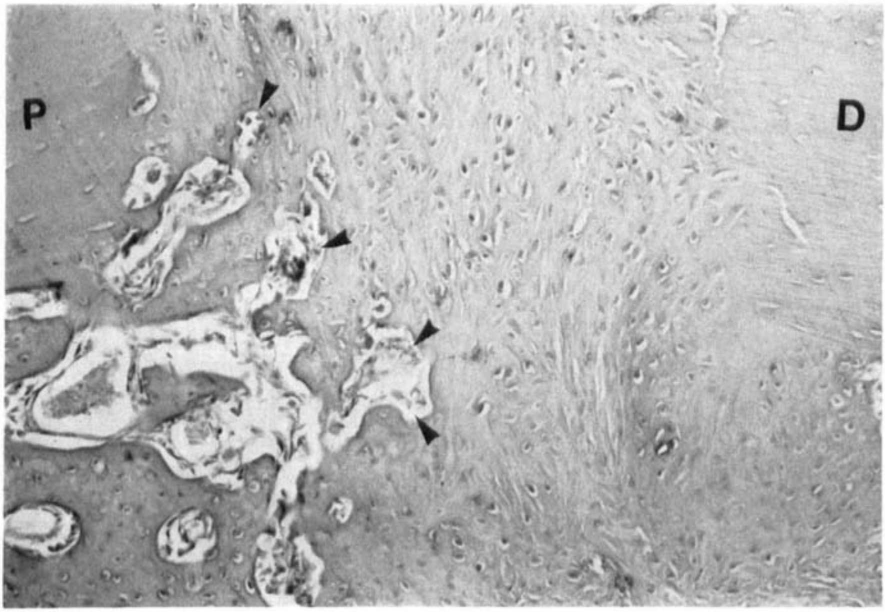

Figure 6. Five weeks after severance, the dead bone-ends are joined, in the ventral zone of their gap, by transversely disposed fibrocartilage, experiencing invasion by bone-forming elements at several sites (arrow). × 167.

did very high-standing bony trabeculae arise. Bony consolidation of the ventral callus, save only very small areas of calcified cartilage, was achieved by Group 1 rats between the 10th and 15th weeks, and between the 7th and 9th by Group 2.

In many animals fibrous connective tissue, fibrocartilage and a form of cartilage with sparsely distributed cells and a pale, non-fibrous, and apparently amorphous matrix were seen in the gap. These three tissues exhibited areas of transition with one another and with the new hyaline cartilage and bone. They also tended to have a particular distribution related to the angulated bone-ends. Fibrous connective tissue usually occupied the wide dorsal aspect, becoming transversely disposed in the narrower ventral region, where it might merge with fibrocartilage. If present, this fibrocartilage also lay transversely and served to unite the osteo-cartilaginous ventral calluses with which it merged (Figure 6). The usual site for the acellular, amorphous cartilage was close to, and fused with, part of the dead bone of the broken margin (Figure 7).

The end-faces early in healing had dead bone undergoing some osteoclastic erosion or lying in contact with young connective tissue cells,

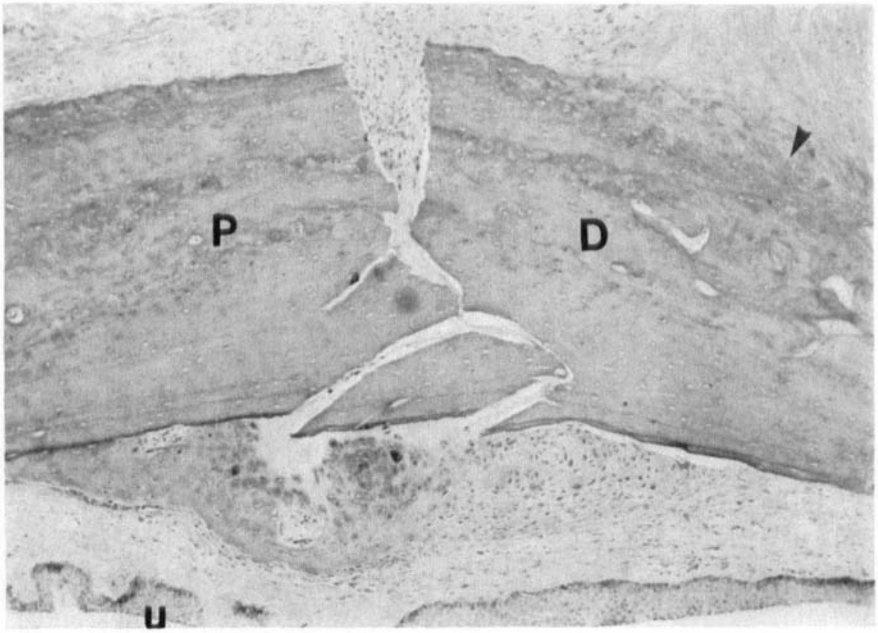

Figure 7. Four weeks after a manual fracture, whose distal position along the shaft is shown in Figure 1. The ventral bridging callus has osteocytes, many chondrocytes, and, near the dead bone, some pale acellular matrix. The end-faces and dorsal shaft surfaces show no callus. The firm attachment of the distal piece to the fibrocartilaginous anterior process is arrowed. × 67.

acellular matrix, or hyaline cartilage near the ventral side of the shaft. The vascular channels opened up in the lesion held osteoclasts (Figure 5) and young connective tissue. Later, a single layer of osteoblasts lined most of the widened channels and some of the end-face and laid down a thin layer of new bone (Figure 2). This seemed to be more a matter of sealing-off of the bone-end from the dense connective tissue of the gap than a significant contribution of callus towards union.

In conclusion, then, the calluses which developed were periosteal, but with a ventral predominance rather than forming symmetrical collars around the bone-ends.

Special Features

In two animals with fibrous union, some islands of urethral epithelium lay within the connective tissue situated between the bone-ends. Neither here nor in any of the other rats did any bone or cartilage form especially close to the epithelium or separated from the calluses (Figure 2).

The young rats were operated upon at a time when endochondral growth was still active along the base of the proximal enlargement. However, when killed, the presence (Figure 4) or absence (Figure 1) of active cartilaginous growth corresponded to that of the controls (Group 3) of similar ages at the time of death. There was no evidence that fracturing the shaft had stimulated endochondral growth at the base or had deferred its cessation.

In contrast to the older group, the young rats developed slightly more callus on the sides of the shaft. The volume of callus in the gap itself was not obviously greater and, as has been mentioned, did not lead to a significantly higher incidence of union.

The bones broken manually differed in their behaviour from that described for the severed os only in the narrowness of the gap, from which fibrocartilage was absent, and the earlier bony replacement of the ventral cartilaginous callus (Figure 7).

Most breaks came in the middle region of the shaft, but several involved the distal part including the tip underlying the distal fibrocartilage. Cartilage cells were regularly observed in the callus tissues in these distal fractures (Figures 1 and 7).

Ossification of Anterior Process

An incidental finding was made in the dense, distal, or anterior process to which the tip of the os priapi attached (Figures 1 and 7). Ruth (1934) and Wiesner (1934) mentioned this structure as being fibrous or fibrocartilaginous. They did not report the basophilia and endochondral ossification substituting in its dorsal region (Figure 8) that were seen here in all the mature rats and most of the control and young rats. The bone formed in the anterior process is irregular in structure with many spaces. No instance of fusion of this bone with the penile bone was noted. There are, therefore, two bony structures in the penis of the mature rat to which the term "os priapi" might be applied.

DISCUSSION

General characteristics. Several general features emerged as characteristic for these fractures of the os priapi. The angulation of the bone pieces with the bulk of the callus forming within the lesser angle, the inconsistent achievement of union, and a union in which transversely

Figure 8. In the distal tip of the mature penis, the fibrocartilaginous anterior process lies above the urethra and is partly replaced in its dorsal region by labyrinthine bone. × 42.

disposed fibrocartilage often participates constitute a picture closer to that described by Pritchard (1964) and Aho (1966) for unstabilized fractures of the rat's tibial shaft than any other experimental fracture reported.

Pritchard (1964) believed that forces from attempted use of the injured limb were important in causing fibrous connective tissue to be transformed to fibrocartilage in the narrow part of the tibial gap. The forces acting on the broken os priapi are unlikely to approach in magnitude those experienced by bones bearing the animal's weight. Nevertheless, some mechanical factor is presumed to have caused the angulation regularly seen, and where the gap between the calluses was very narrow, that factor may have resulted in some transformation of fibroblastic tissue into fibrocartilage.

Union. Ignoring the controversial question of whether or not connective, cartilaginous, and osseous tissues can, in the transition zones observed, slowly transform one into another, the process of tissue replacement undoubtedly plays a role in fracture repair. Callus hyaline cartilage certainly is replaced by bone, and fibrocartilage may be so replaced. An hypothesis of, perhaps, general application can be proposed: any cartilage tissue bridging a fracture gap will eventually be replaced by bone in endochondral ossification. In particular, this is probable in the rat's penis, since the distal fibrocartilage already described regularly undergoes some replacement ossification in the unoperated rat. Fibrocartilage linking penile fractured bone-ends would be expected to be at least as prone to ossification as that structure.

Assuming the eventual substitution of bone for cartilage, a fracture may be deemed united when it is linked by a continuous band of tissues containing osteocytes or chondrocytes. Union so defined histologically occurred in twenty-eight of forty-five animals with severed bones surviving for two weeks or longer. This incidence of minimal union, the narrowness of the gaps, around ½ mm, sometimes remaining ununited, and the relatively long period for bony consolidation of the callus tissues mark the os priapi as a site of poor bony healing.

The rat unaided can bridge with bone gaps of 1 mm in the fibula and 2 mm in the rib (Mulholland & Pritchard 1959), but only 0.1 mm in the skull vault (Pritchard 1946). The widest gaps united by bone or cartilage in the os priapi were approximately 1 mm across, but gaps of half that size sometimes remained fibrous. The penile bone therefore falls between the skull vault and the long bones of the skeleton in its powers of regeneration.

Internal callus. One possible contributor to this poor repair is the lack of an endosteal or internal callus derived from a marrow cavity. Vascular channels opened up by the cut were eroded and then a thin overlay of new bone appeared in them and on the dead end-face. The shaft end-faces thus behave like injured skeletal cortical bone (Pritchard 1964).

Several authors stress the major contribution made to bony healing by the internal callus when cortical drill holes penetrate to the marrow cavity of long bones (Ely 1927, Bourne 1942, Melcher & Irving 1962). Enneking (1948) went further by maintaining that the internal callus was more important than the external for the repair of complete tibial shaft fractures in rats. What occasions the vigorous internal callus in fractures and penetrating holes in long bones and is deficient in the marrow-less shaft of the os priapi may be: *space* for abundant, internal, bone formation, or numerous, marrow, *osteoprogenitor cells*, or both these factors.

Fibrous situation and age. Another factor acting against bony union may be the dense fibrous situation in which the os lies, which could predispose to fibrous union. One way in which this might happen is that, by inserting into the shaft at various points, the fibrous tissue is so firmly attached that the periosteal reaction is restricted. Periosteal reaction might be limited because of little stimulation from tearing or because of the lack of space in which high bony trabeculae could grow. The lesser callus formed on the dorsal surface of the shaft may, in part, reflect a firmer attachment of the periosteum there than to ventral surfaces.

The periosteal reaction along the shaft sides was somewhat greater in the younger animals, which may be related to the young periosteum's being less firmly attached to surrounding connective tissues. Aho (1966) compared the repair of unstabilized tibial fractures in young and old rats and noted somewhat quicker and a little greater production of callus in younger animals. In the penile bone the amount of callus produced is less in both young and old animals than in the tibia and no significant difference in callus volumes was apparent. Moreover, the penile fracture involved the shaft which even in these young animals was not growing very actively. The bone actually broken may be relatively more mature than the base of the os and other growing zones of such young rats.

The firmness with which the broken os remains attached to its fibrous surroundings and its lack of weight-bearing may explain why fracture does not stimulate or prolong endochondral growth at the proximal base in the manner reported by Bisgard (1936) for the epiphyses of the growing goat's tibia when the shaft is broken.

Cartilage in callus. No matter where along the length of the shaft the cut or break came, the events of fracture repair were the same and, in particular, cartilage cells were always present in the callus. They occur in spite of the description by Ruth (1934) of the distal part of the shaft as originating without the prior formation of cartilage, although no precise proportion of the shaft's length arising in this non-endochondral way could be given.

The hypothesis that bones developing intramembranously do not develop cartilage during fracture healing was disproved by Pritchard's (1946) solitary observation in a rat skull, the fibrocartilage seen in healing fractures of the rat mandible by Sarnat & Schour (1944), and the cartilage and endochondral ossification noted by Richman & Laskin (1964) in one-fourth of fractures involving the dog's zygomaxillary complex. Scraping the skull before fracture makes cartilage more likely to form in infant rats because, Girgis & Pritchard (1958) believe, the ischaemic environment thus produced favours the formation of cartilage. Contact and compression between bone pieces (Eggers, Shindler & Pomerat 1949) and vitamin-A deficiency (Beresford, unpublished observation) also promote the development of cartilage in healing rat parietal bones. Factors existing or arising at the time of fracture may therefore prevail over any tendency of callus cells to form only bone because of their embryonic origin or associations.

Epithelial bone-induction. Because of the well-known, osteogenic, inductive power of urinary tract epithelium, the greater development of callus on the urethral side of the os might be another manifestation of this phenomenon. Several arguments are against such an induction's having taken place. New bone and cartilage were always in continuity with the old bone and were separated by a substantial layer of connective tissue from the epithelium. The urethral epithelium at this point is not transitional and has not been shown experimentally to have any osteogenic influence. Finally, the rat's urinary bladder epithelium regularly induces bone formation, but the amount formed is very small in comparison with, for example, the guinea-pig's (Beresford & Hancox 1967).

Bone in human phallus. This investigation has no direct bearing on any orthopaedic problem involving bone in the human penis, since bone and cartilage are an abnormality in man's phallus. Eglitis (1953) reviewed five, previous, histologically confirmed reports of bone in the human penis and added another himself. He drew attention to a number of considerations indicating that the bone's occurrence was a pathological event of the same nature as the formation of metaplastic bone in the connective tissue of many other organs of the body, and was in no way to be construed as an atavistic attempt to form an os priapi of specific shape, size, and position such as other primates possess (Gerhardt 1910).

SUMMARY

Fractures of the shaft of the penile bone were made by severance or closed manipulation in 66 young and mature rats. Healing was followed histologically for a period from 4 to 210 days after operation. Union of the angulated bone pieces by bone or cartilage occurred in just over half the severed bones and in all 12 manual fractures. Although six severed gaps 1 mm wide were bridged, others of only ½ mm width remained un-united in animals surviving for two weeks and longer.

No clear difference in the volume of callus was noted between the

young and mature groups. The callus was osteocartilaginous and of periosteal origin. It formed mostly inside the angle on the ventral, urethral sides of the shaft. An internal callus was not formed, probably because the marrow cavity of the base does not extend along the shaft.

The fibrocartilaginous anterior process lying distal to the penile bone was found regularly to undergo some endochondral ossification unrelated to any fracture in the principal penile bone of these and 10 intact rats.

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