J. Anat. (1988), **158**, pp. 173–187
With 15 *figures*
Printed in Great Britain

Periosteal structure and development in a rat caudal vertebra

GRAHAM ELLENDER, SOPHIE A. FEIK
AND BARBARA J. CARACH

Department of Preventive and Community Dentistry, University of Melbourne,
711 *Elizabeth Street, Melbourne, Victoria* 3000, *Australia*

(*Accepted* 16 *September* 1987)

INTRODUCTION

Mechanical forces transmitted through the soft tissues are implicated as direct stimuli of bone remodelling (Lutfi, 1974; Lanyon, 1982); however, the modulatory mechanisms involved are not clear. Recent work emphasises the role of periosteum and adjacent soft tissues in bone remodelling (Chong & Evans, 1982; Pollard, Feik & Storey, 1984; Storey & Feik, 1985). These latter studies have shown clearly that tension transmitted to the bone surface by way of the periosteum induces bone formation while forces resolved into pressure/compression by periosteum on the bone surface evoke resorption.

The rat tail (Storey, 1981) has provided a suitable model for the study of bone and periosteum during development, transplantation and stress application (Feik & Storey, 1983; Pollard *et al.* 1984; Storey & Feik, 1982, 1985, 1986). A major advantage of this model, besides the accessibility of the bones, is the capacity to apply mechanical stress without surgical injury to the region under investigation.

To understand the modulatory role of periosteum, it is essential to have a detailed knowledge of its structure and its relationship and interaction with both bone and the surrounding soft tissues; firstly, during normal development and then in mechanically stressed situations. Few systematic studies have been undertaken into these structural features.

Periosteum is regarded generally as having two basic layers – an outer fibrous and an inner cambial or osteogenic layer (Ham, 1974). However, some workers describe a zone of transition between the two layers characterised by a rich network of capillaries (Hamilton, Boyd & Mossman, 1962). An ultrastructural study (Tonna, 1974) describes changes in the periosteum with age. There appear, however, to be no systematic studies of periosteal structure and ultrastructure in a consistent and defined site throughout growth, development and maturation and in response to specific stimuli.

Since the morphology of the periosteum varies under these conditions (Chong, Evans & Heeley, 1982) it is fundamental that the structure of periosteum at a specific site be defined during development. This paper describes the periosteum of the rat tail at a reproducible reference site and provides a basis for further studies of the reaction of periosteum to mechanical stress. In this paper emphasis will be placed on the 50 g rat as this model is used in parallel studies of the effects of mechanical stress on the periosteum (in preparation); caudal vertebrae at this stage of development are an excellent model for the study of remodelling as the active skeletal tissues (bone and periosteum) are more responsive than are those of the skeletally mature adult.

MATERIALS AND METHODS

Female Sprague–Dawley rats were housed at 22 °C and fed Barastoc pellets (Melbourne, Victoria) and water *ad libitum*. Age and mass data were obtained at intervals from birth to 300 days from 12 rats selected at random from within the established colony. The means and standard deviations of their mass within the groups were calculated and a growth curve plotted. Additional animals, anaesthetised with chloral hydrate (36 mg/100 g body mass), were used for experimental purposes in groups of seven rats of 10, 20, 35, 50, 70, 90, 120, 150, 200 and 250 g mass. They were radiographed to identify and measure the fourteenth caudal vertebrae (CV14). All radiographs were taken on a Faxitron 43805N X-ray System Unit (Hewlett-Packard Co., McMinnville, Oregon) with a current of 2·5 mA and a voltage of 23–27 kVp on Kodak Industrex M film. The target-film distance was 610 mm. Measurements were made on the radiographs of the length between articular surfaces and the mean maximum width of the epiphyses of CV14 and the width/length ratio plotted against age.

Scanning electron microscopy

The CV14 of two 50 g rats were macerated in tap water at 37 °C until defleshed, soaked in 1 % sodium hypochlorite solution for 15 minutes, dehydrated in ascending concentrations of ethanol, cleared through epoxypropane and subsequently infiltrated with camphene and dried by sublimation (Watters & Buck, 1971). These specimens were sputter coated with gold and examined in a Siemens Autoscan scanning electron microscope.

Histology

The radiographed tails were skinned and segments containing CV13–15 were severed and fixed in neutral buffered formalin. Tissue was decalcified in a solution of 3·4 % sodium formate and 17·5 % formic acid. Transverse and longitudinal 5 μm sections were cut through the middle of CV14 and stained with Ehrlich's haematoxylin and eosin-phloxin, picrosirius red (Junqueira, Bignolas & Brentani, 1979) and Masson's trichrome. On both transverse and longitudinal sections of CV14 the width of the periosteum was measured using a vernier eyepiece graticule and the results plotted against age.

Transmission electron microscopy

Tails of anaesthetised rats of 10–35 g mass (n = 15) were skinned and immersed in Karnovsky's fixative (Karnovsky, 1965). The CV14 was excised *in toto* under immersion and fixed for a further two hours at room temperature in fresh fixative.

Anaesthetised rats of 50 g and larger were perfused by intracardiac puncture with heparinised 0·1 M cacodylate buffer pH 7·3 followed by Karnovsky's fixative at room temperature for five minutes. The tail was skinned and CV14 excised and immersed for one hour in fixative. The vertebrae were cut transversely through the mid-diaphysis into 1 mm slices and these were immersed for a further hour in the fixative. One series of tissues, representing each of the specified masses, was demineralised at room temperature in 0·1 M EDTA containing 5 % glutaraldehyde pH 7·3 (Baird, Winborn & Bockman, 1967). Tissues were postfixed in 2 % osmium tetroxide in 0·1 M cacodylate buffer, pH 7·3, for two hours at room temperature and block-stained in 0·5 % uranyl acetate. They were dehydrated through graded acetones and processed to Spurr's hard formulation resin (Spurr, 1969) through a protracted infiltration sequence. Semithin (1 μm) survey sections were stained with toluidine blue, and ultrathin sections

(70–80 nm thickness) cut of the ventrolateral surface of the mid-diaphysis. Sections were collected on 400 or 600 mesh slim bar grids (Gilder, Bartelt Instruments Pty. Ltd, Melbourne, Victoria), stained with 2% aqueous uranyl acetate followed by Reynolds' lead citrate (Reynolds, 1963), and examined in a Philips EM300 transmission electron microscope.

RESULTS

Growth data

The postnatal growth curve (Fig. 1) of the female Sprague–Dawley rat is sigmoid and shows three phases. Firstly, a developmental phase is seen from birth to 15 days (30 g) during which time there is a slight acceleration in growth velocity; this period coincides with the development of motor skills. This is followed by a rapid growth phase to around 50 days with a slowing by 70–80 days. The third is the maturation phase during which mass increases slowly and skeletal maturity is attained.

Caudal vertebrae develop from cartilage *anlagen* and in the early stages the presumptive bones are broader than they are long (Fig. 2). As development proceeds the caudal vertebrae lengthen in proportion to their width; the width/length ratio decreases initially and attains a constant value at maturity.

Scanning electron microscopy

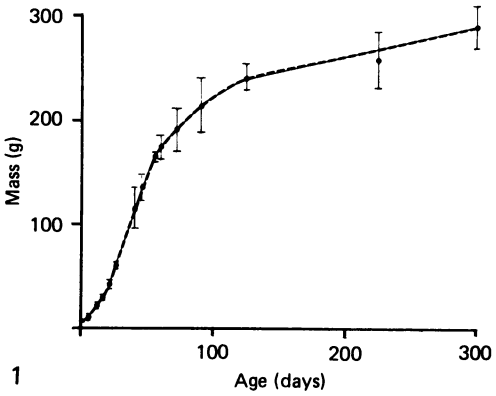
The CV14 of the 50 g rat is a dumb-bell shaped bone with symmetrical lateral aspects (Fig. 3*a*) but asymmetric dorsoventrally (Fig. 3*b*), having a more pronounced concavity in the mid-diaphysis of the ventral surface where the vascular plexus is located. The ventral surface contains many vascular foramina and no bony processes. Reduced zygapophyses are present on the dorsal surface while on the lateral surface transverse processes are more prominent near the epiphyses and minimal towards the mid-diaphysis.

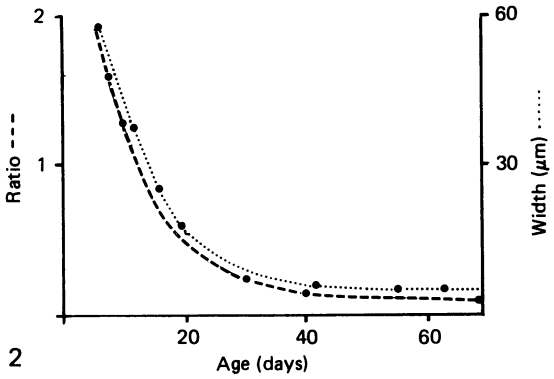
Histology

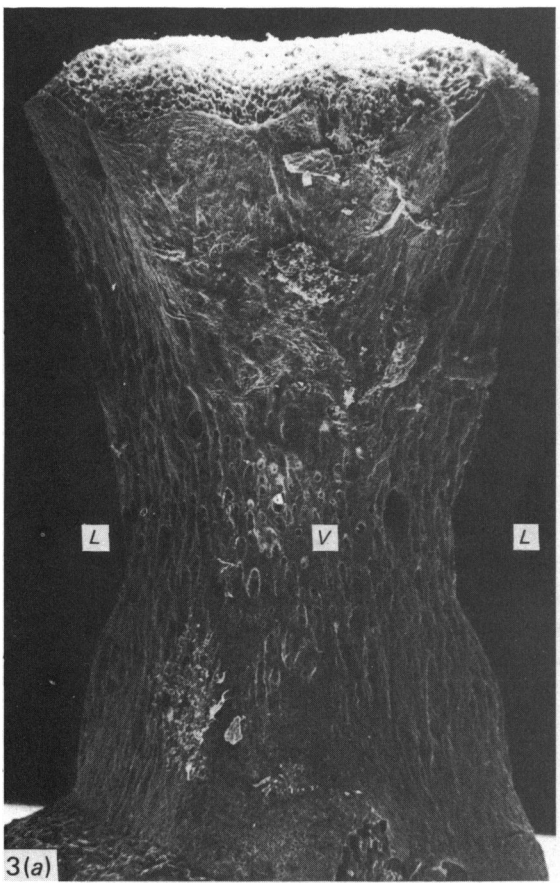
Longitudinal sections through the ventrolateral plane of CV14 of the 50 g rat show small vessels in the mid-diaphyseal concavity at some distance from the muscle bundles (Fig. 4).

Transverse sections (Fig. 5*a*) show that the ventrolateral surfaces lie more nearly parallel to the sagittal axis than do the dorsolateral surfaces. The bone is surrounded by four muscle and ligamentous bundles which are symmetrically disposed but are of unequal size, the ventral bundles being larger than the dorsal ones. The fasciae encasing these bundles attach to the fibrous periosteum at the bony processes and divide around the ventral vascular plexus. The 10 g (Fig 5*b*) and 250 g (Fig. 5*c*) vertebrae show essentially the same soft tissue/bone relationships but the structure of the bony shaft and the thickness of the periosteum differ, The periosteum in the mid-diaphysis is approximately 60 μm in the 10 g rat and is reduced to approximately 5 μm in the 250 g rat (Fig. 2). The curve closely reflects that of the width/length ratio of the CV versus age.

The morphology of the periosteum changes throughout development. During the rapid growth phase from approximately 35–110 g two layers can be delineated by histology (Fig. 6*a*). The deeper osteogenic or cambial layer adjoining the bone consists at this stage of a row of plump osteoblasts while the superficial layer of the fibrous periosteum contains spindle-shaped fibroblasts and bundles of collagen aligned predominantly parallel to the bone surface (Fig.6*b*). In the early developmental phase







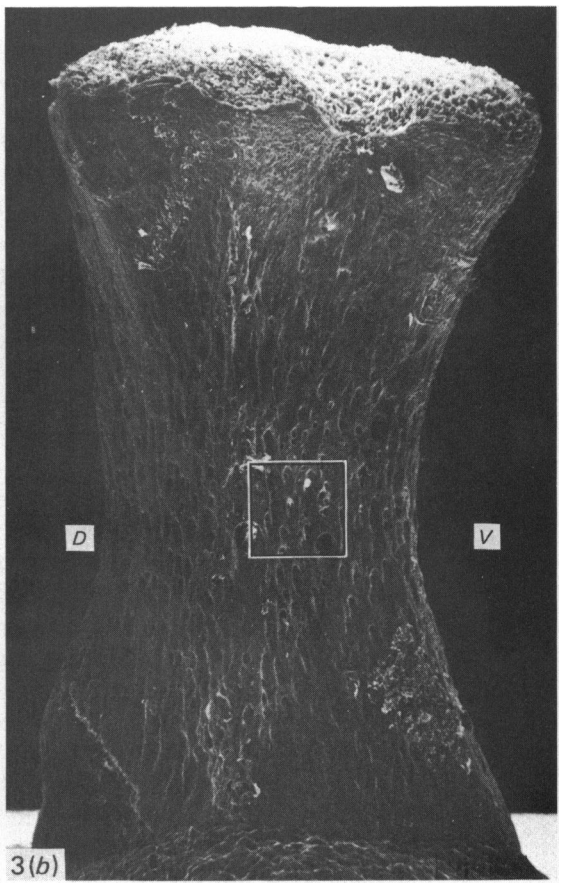


Fig. 1. Cross sectional mass data of groups (n = 12) of female Sprague–Dawley rats showing a sigmoid postnatal growth curve.

Fig. 2. Ratio of the maximum epiphyseal width to total length of CV14 plotted against age in days (---). Mid-diaphyseal periosteal width of CV14 versus age (···). The curves are closely concurrent.

Fig. 3(*a–b*). SEM of CV14 from a 50 g rat. (*a*) The CV has symmetrical lateral sides (*L*) and numerous vascular foramina on the ventral surface (*V*). (*b*) The ventral aspect (*V*) shows a more pronounced mid-diaphyseal concavity than the dorsal (*D*). The area of interest is outlined. × 50.

at 10 g (Fig. 6*c*), when at its widest, the periosteum appears to be still differentiating and is not as well structured as in the later stages. Osteoblasts are irregularly arranged and elongated and the fibrous periosteum is at its widest and least compact and is interspersed with plump, active fibroblasts. With maturity the cellular and fibrous layers of the periosteum become more consolidated. The osteogenic cells become increasingly spindle-shaped with a reduced cytoplasmic volume. By full maturity, 250 g (Fig. 6*d*), they comprise a narrow lining barely visible in some areas, covered by a thin fibrous layer.

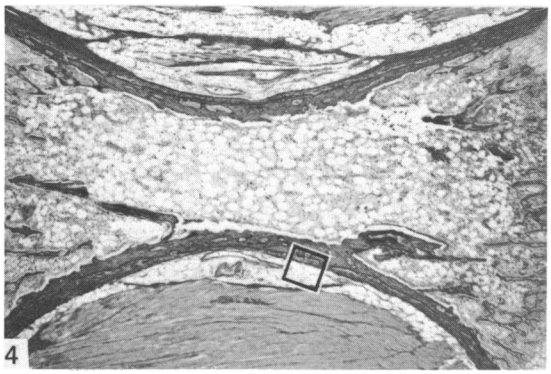
Transmission electron microscopy

Transmission electron microscopy is limited to the ventrolateral surface of the mid-diaphysis adjacent to the vessels (Fig. 4). These results are presented in chronological sequence.

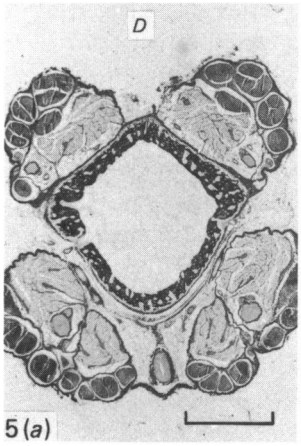
At 10 g, the periosteum is differentiating into three layers (Fig. 7). The cells adjacent to the osteoid and bone resemble functional osteoblasts but are elongated rather than cuboidal; the rough endoplasmic reticulum (RER) is not dilated generally nor is the Golgi zone prominent. Relatively few cell processes enter the osteoid. Areas of cellular degeneration characterised by vacuolation and diverse mitochondrial configurations are seen occasionally. The cellular periosteum may be one or several cells thick and merges with the mid-zone. This middle zone contains many undifferentiated cells which have a large nuclear to cytoplasmic ratio, little condensed chromatin, sparse RER, few organelles and many processes which often contact nearby cells (Fig. 8). Vessels are not prominent at this stage. The collagenous matrix is sparse and the fibres present are aligned generally parallel to the long axis of the bone. Sharpey-like perpendicular bundles of fibres penetrate the bone. In the outer superficial zone – the fibrous periosteum – there are many layers of fibroblasts which have little cytoplasm and large nuclei with dispersed chromatin. Cells in this layer are more closely associated than those in the mid-zone; mitoses are seen occasionally. The collagenous matrix is more abundant and organised into bundles; small clusters of microfibrils, reminiscent of elastin precursors, are visible although elastin as such is absent.

At 20 g the periosteum does not differ significantly from that of the 10 g rat. The osteoblasts although still elongated have dilated RER with dense cisternal contents and a developed Golgi zone. Vascular elements (Fig. 9) have developed in the loose connective tissue of the mid-zone. The periosteum of the 35 g rat, although thinner, shows greater organisation. The osteoblasts are plumper, appear to be actively synthesising and some contain glycogen granules. More cytoplasmic processes extend into the osteoid and some early osteocytes are being incorporated.

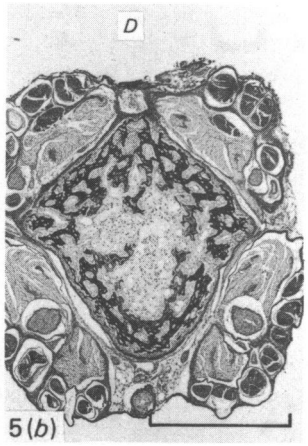
In the 50 g rat the periosteum has differentiated into three distinct zones (Fig. 10): an inner osteogenic zone, a vascular undifferentiated mid-zone and an outer fibrous zone. The osteogenic layer comprises a single layer of cuboidal osteoblasts with prominent nucleoli and peripheral condensed chromatin in oval nuclei. The mitochondria in some osteoblasts display a variety of forms, some showing swelling and others condensation; mitochondria in immediately adjacent cells appear normal. Numerous cytoplasmic processes project into the osteoid along with Sharpey fibre bundles. These bundles, composed of densely packed collagen fibrils of unimodal diameter (Fig. 11), originate deep in the bone, traverse the osteogenic layer and insert into the more superficial periosteum. Pre-osteoblasts delineate the inner zone from the mid-zone. The mid-zone (Fig. 10) contains many small blood vessels, mononuclear phagocytes and undifferentiated cells. The collagenous matrix is more abundant than in the earlier stages but is still less dense, with more ground substance than the adjoining fibrous periosteum. The superficial fibroblasts are spindle-shaped and the



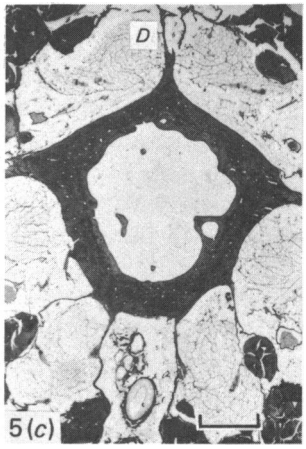
4



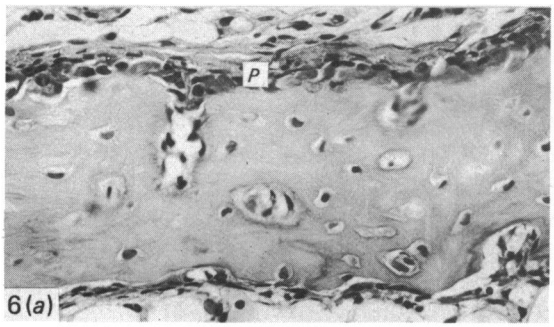
5 (*a*)



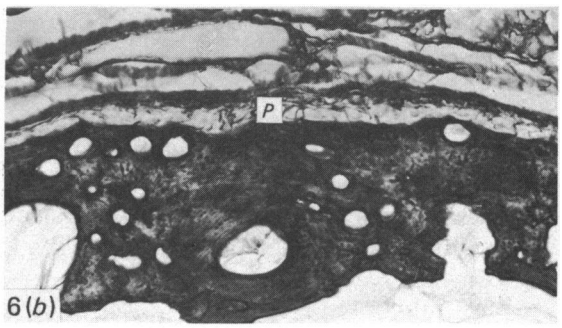
5 (*b*)



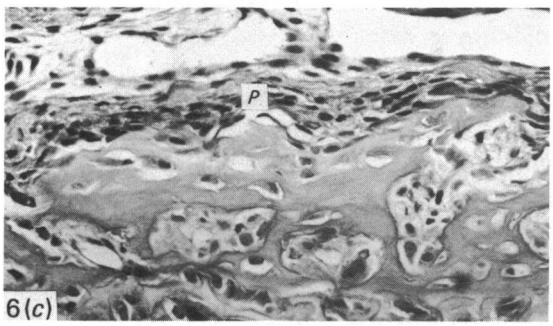
5 (*c*)



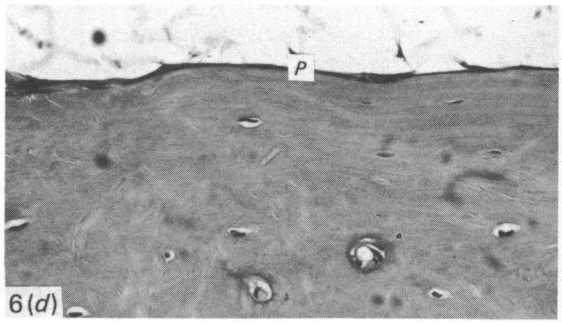
6 (*a*)



6 (*b*)



6 (*c*)



6 (*d*)

nuclei contain more heterochromatin than previously; cytoplasm is scant and frequently drawn out into long cytoplasmic processes which connect with other fibroblasts via gap junctions (Fig. 12) to form a syncytial structure. The cells are less closely packed than those in the younger animals and the matrix consists of densely packed collagen fibrils of relatively uniform diameter interspersed with some elastic fibres which are first clearly evident at this stage (Fig. 13). The elastic fibres lie along the long axis of the tail parallel to the predominant alignment of the adjacent collagen fibrils.

The 70 and 90 g periosteum has a structure similar to that of the 50 g rat but with a reduction in overall periosteal thickness, mainly at the expense of the mid-zone. The matrix of the fibrous periosteum is more densely packed. By 120 g there is further reduction in periosteal thickness, although the mid-zone with its vascular elements is still recognisable.

At 150 g (Fig. 14) the mid-zone can barely be distinguished and cellular debris now marks its position. The osteoblasts are still active but are usually elongated rather than cuboidal; only occasionally are pre-osteoblasts seen. Extraperiosteal vessels encroach onto the fibrous periosteum which contains fibrocytes with long thin cytoplasmic processes. As the rat matures there is a progressive involution of the periosteum so that by 250 g (Fig. 15) the cellular periosteum consists of a layer of flattened and greatly elongated osteoblasts with decreased RER adjacent to an osteoid seam of noticeably reduced thickness. There is no discernible mid-zone and the fibrous periosteum contains a few fibrocytes with long thin connecting processes.

DISCUSSION

This study considers the relationship of the enveloping soft tissues to bone during normal growth, development and maturation of the fourteenth caudal vertebra of the rat tail. The gross and functional anatomy of the caudal vertebrae is reviewed in particular relation to the bone/soft tissue interaction. Knowledge of the structure of periosteum under normal functional conditions is necessary prior to ultrastructural studies of the stressed periosteum using the same experimental model.

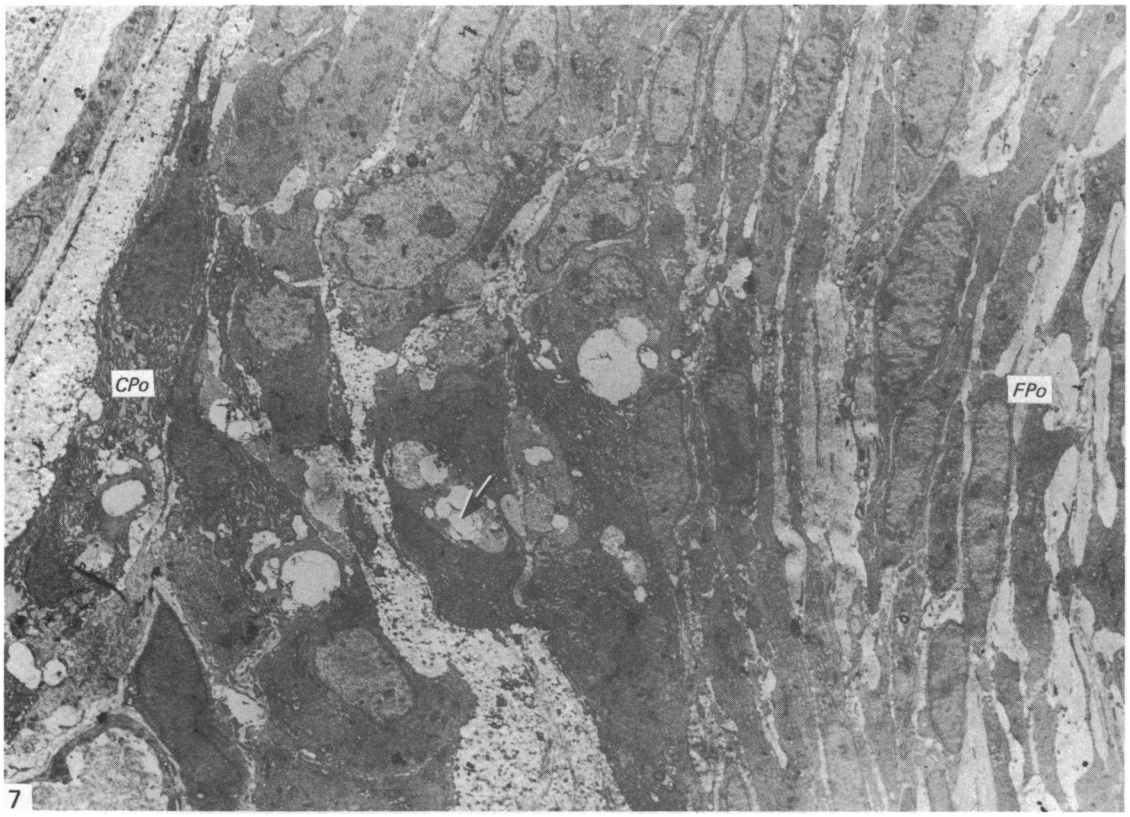
Periosteal morphology not only varies with age but also between areas of the same bone (Chong *et al.* 1982). Therefore it is essential that a specific well-defined anatomical site be selected. A previous study of age changes in the mouse periosteum lacked a distinct site of reference and hence is difficult to interpret. The present study focuses on the ventrolateral surface of the mid-diaphysis of CV14 in rats of differing masses. This specific site is the subject of further ultrastructural investigations on the reactions of stressed periosteum (to be reported). Lying more nearly parallel to the

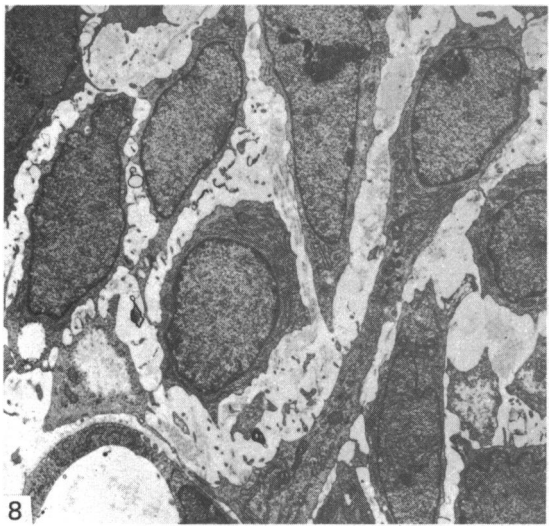
---

Fig. 4. Histology of longitudinal section through the ventrolateral plane of CV14 from a 50 g rat. The area outlined adjacent to the vessels is that used for the ultrastructural studies. Masson's trichrome. × 26.

Fig. 5(*a–c*). Histology of transverse sections through the mid-diaphysis of CV14. The dorsal (*D*) muscle and ligamentous bundles are smaller than those on the ventral surface where the main vascular plexus is located. Bar = 500 μm. Picrosirius red. (*a*) 50 g. × 23. (*b*). 10 g. × 37. (*c*) 250 g. × 14.

Fig. 6(*a–d*). Histology of mid-diaphyseal transverse sections of CV14 showing the variation in the structure and width of the periosteum (*P*) with age. (*a*) 50 g. Two layers only can be distinguished histologically. H and E. (*b*) 50 g. Some collagen bundles arise from the bone and traverse the periosteum. Picrosirius red. (*c*) 10 g. The periosteum is at its maximum width but the layers are not as clearly delineated as at 50 g. H and E. (*d*) 250 g. There is a marked reduction in both the cellular and fibrous layers of the periosteum. H and E. × 260.





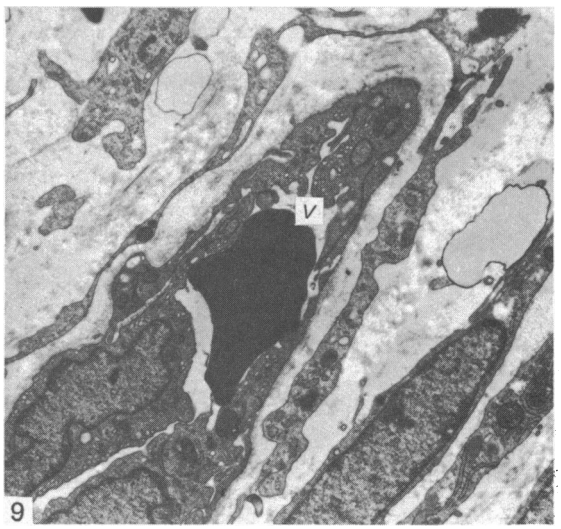
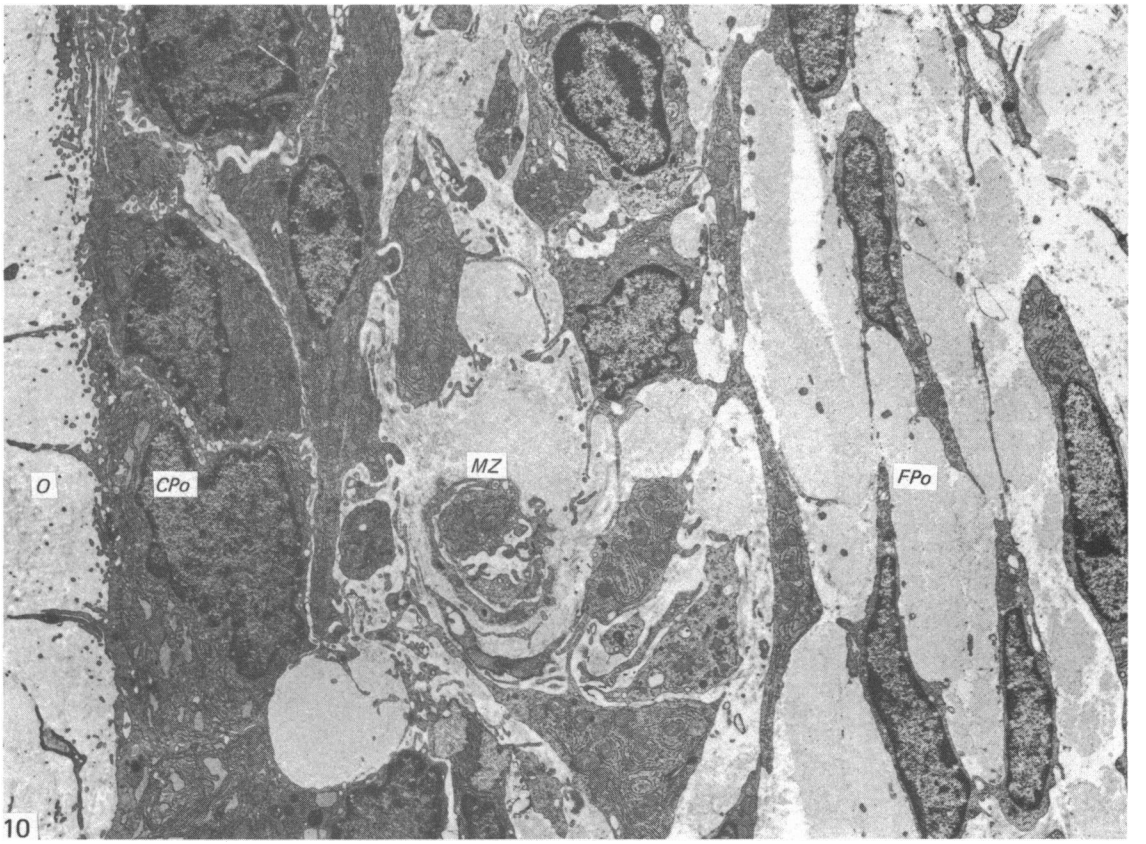
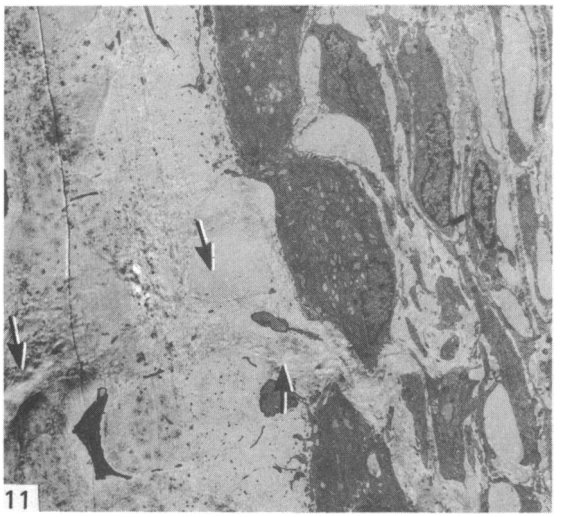


Fig. 7. TEM of periosteum at 10 g. Elongated functional osteoblasts comprise the cellular periosteum (*CPo*). Some cells show areas of degeneration (arrow). The fibrous periosteum (*FPo*) contains closely packed fibroblasts. × 2750.

Fig. 8. TEM of mid-zone of the periosteum at 10 g. Undifferentiated cells with many contacting cell processes are separated by a sparse collagenous matrix. × 5625.

Fig. 9. TEM of mid-zone of the periosteum at 20 g illustrates the development of vessels (*V*) at this stage. × 7050.





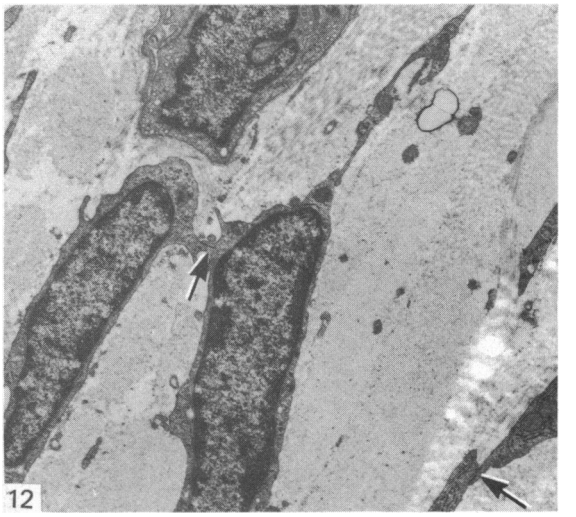
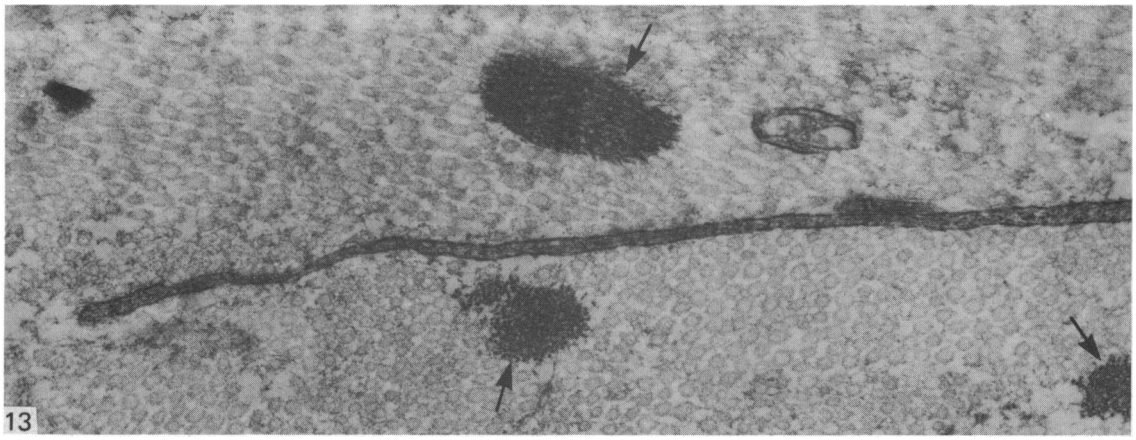


Fig. 10. TEM of the periosteum at 50 g has three discernible layers. The cellular periosteum (*CPo*) consists of a row of osteoblasts with many processes extending into the osteoid (*O*). Pre-osteoblasts delineate this zone from the mid-zone (*MZ*) in which are vessels, undifferentiated cells and mononuclear phagocytic cells in a loose collagenous matrix. A denser matrix and fibroblasts in a syncytial arrangement characterise the fibrous periosteum (*FPo*). × 5375.

Fig. 11. TEM of periosteum at 50 g. Dense collagenous Sharpey fibre bundles (arrows) extend from the bone surface and osteoid layer into the periosteum. × 2300.

Fig. 12. TEM of fibrous periosteum at 50 g. Fibroblasts frequently make contact with adjoining fibroblasts via gap junctions (arrows). × 8000.



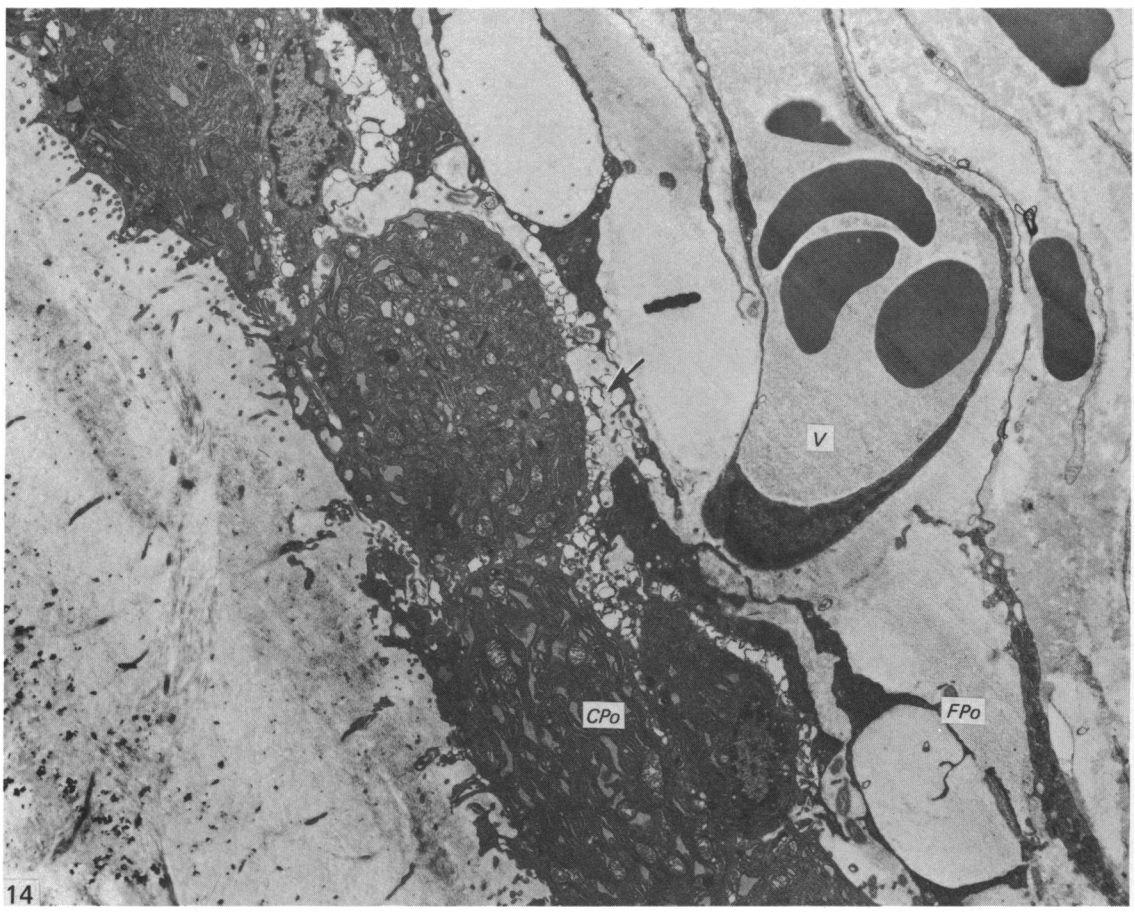


Fig. 13. TEM of fibrous periosteum at 50 g. Elastin bundles (arrows) are orientated in a direction similar to that of the surrounding collagen fibrils. These fibrils are closely packed and of relatively uniform diameter. × 27550.

Fig. 14. TEM of periosteum at 150 g. Extraperiosteal vessels (*V*) encroach onto the fibrous periosteum (*FPo*). Cellular debris (arrow), adjacent to the osteoblasts in the cellular periosteum (*CPo*), marks the position of the mid-zone. × 4600.

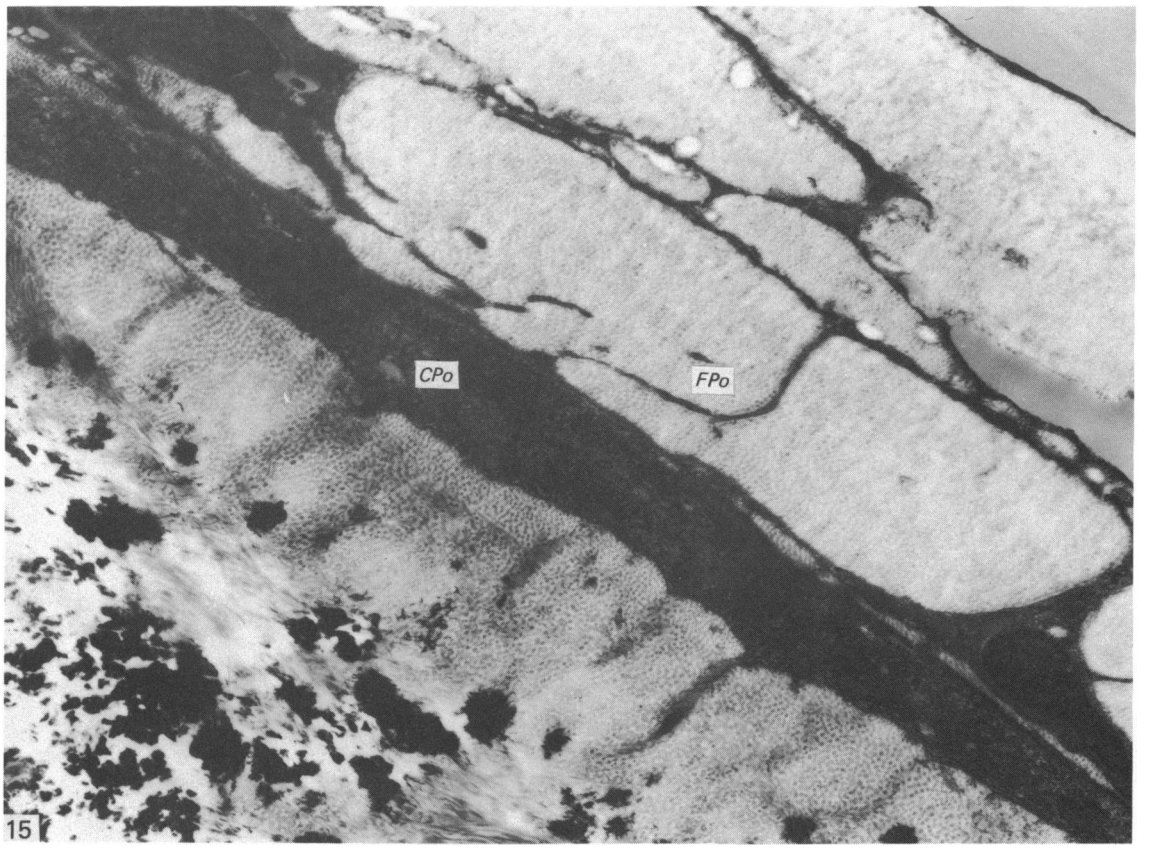


Fig. 15. TEM of periosteum at 250 g. The cellular periosteum (*CPo*) consists of greatly elongated, flattened osteoblasts. A syncytial arrangement of fibroblasts is seen in the fibrous periosteum (*FPo*). × 20375.

sagittal axis, periosteum of the ventrolateral surface is subjected to less shear force than is that on the dorsolateral, in function and on the application of mechanical stress. When the tail is mechanically stressed in the lateral direction the responses in the periosteum in the two regions can be examined – one being predominantly influenced by compression of the osteogenic layer and the other by tension. CV14 was selected since it has been used in previous remodelling studies (Storey & Feik, 1985).

The growth of the rat follows a sigmoid curve. The reduced growth rate during the developmental phase from birth to 15 days coincides with the emergence of postural and locomotor skills (Altman & Sudarshan, 1975). Maximum growth occurs between 15 and 70 days during which the body mass increases from 40 to 150 g. The slowing of growth at 70–80 days is consistent with a previous report of the female Sprague–Dawley rat (Acheson, MacIntyre & Oldham, 1959) and indicates the onset of skeletal maturity. Because rats are skeletally immature at birth, as is man, they provide a good model for studying the response of skeletal development to environmental stress (Acheson *et al.* 1959). The development of the diaphysis is influenced by cartilage growth via the perichondrium/periosteum (Lutfi, 1974). As the cartilage expands the periosteum is stretched and moves away from the bone surface and increased tension on the periosteal fibres promotes subperiosteal bone deposition

(Harkness & Trotter, 1978), a phenomenon which has been observed also in mechanically stressed bones (Storey & Feik, 1982, 1985).

The progressive thinning of the periosteum closely reflects the decrease in the width/length ratio of the caudal vertebrae with development. Since the periosteum exerts a restraining effect on growth (Crilly, 1972; Harkness & Trotter, 1978; Warrell & Taylor, 1979) changes in its structure, and therefore physical and functional properties, must be mirrored by changes in bone form. This is seen both in normal development and in transplants (Feik & Storey, 1983) where the bones become longer relative to width as growth proceeds. In addition, since the periosteum appears to behave as an elastic membrane (Harkness & Trotter, 1978) in young bones, the changes in structure with age, especially the reduction of the fibrous layer, may result in a decrease in elasticity which in turn restricts the lateral growth of cartilage.

The CV14 has symmetrical lateral sides but asymmetrical dorsal and ventral surfaces. The ventral surface has a more accentuated mid-diaphyseal concavity than the dorsal surface. The shape of the CV probably reflects the biomechanical forces exerted on the bone by the muscles and ligaments. The muscle bundles on the dorsal surface function to maintain the horizontal posture of the tail and, in so doing, are maintained under constant tension. This is reflected in the shape of the vertebra. However, when the extent of muscle and ligament development is considered, there is an apparent paradox since there is greater development of the ventral than of the continually tensed dorsal bundles. This can be explained by postulating that the dorsal bundles have a purely suspensory role while the ventral ones are responsible for control of lateral movements and for trim of the elevation of the tail by antagonism.

The present study of the rat tail periosteum during growth, development and maturation demonstrates that the periosteum is basically a three layered structure. The composition and prominence of these layers change markedly during the life cycle of the rat. An understanding of these structural changes and their relation to function is fundamental when considering growth, remodelling and repair of bone.

The ultrastructure of the cells in the inner cambial layer closely reflects the general growth pattern. It the earliest stages the osteoblasts are elongated and not highly active; during the period of maximum somatic growth they become cuboidal and the appearance of the cytoplasmic organelles is consistent with rapid protein synthesis and secretion. The range of mitochondrial forms at this stage is not a fixation artefact and probably reflects functional changes in normal active cells (Ord & Smith, 1982). More osteoblast processes enter the osteoid during this period which coincides with the plateau attained in the width/length ratio of the caudal vertebra. With a marked differential of growth in width and length and consequent shearing of the periosteum it may not be feasible to establish a firm connection between bone and periosteum via the cellular processes; as stability is reached the number of these processes increases. When body growth slows the inner layer becomes reduced to a thin lining of flattened, elongated and quiescent cells.

The mid-zone similarly changes during development. During the developmental phase it is poorly differentiated although relatively wide and contains few vessels. It is clearly seen and attains its maximum development during the rapid growth phase. With maturity it involutes and is absent in older animals. The undifferentiated cells which comprise the bulk of the cellular element are thought to provide the progenitor cells for both the osteoblastic and fibrous layers. A few mononuclear phagocytes are present in the mid-zone and are likely to have a role in the local control of bone remodelling (Baron, Vignery & Horowitz, 1984). The collagen of the extracellular matrix is moderately well ordered. Its structure is suited to a supporting role and, in

conjunction with the non-collagenous matrix components, could have a visco-elastic function which buffers the underlying osteogenic layer against stress fluctuations within the physiological range. Further, the dispersed nature of the mid-zone enables effective transfer of nutrients and metabolites to and from the osteogenic layer during active growth. The loss of the mid-zone with age appears to be a functional adaptation and its potential to reform, following trauma or with increased stress, requires detailed investigation.

The fibrous periosteum in the early stages consists of a loose connective tissue matrix with active fibroblasts; its function at this stage is formative rather than supportive, for the tail is short and stumpy. With increase in length of the tail the fibroblasts in this layer develop a syncytial arrangement within the dense collagenous matrix. The collagen fibrils are aligned predominantly in the long axis of the tail along the direction of maximum tension. The elastin component of the matrix appears during the maximum growth phase, being preceded by collections of microfibrils which are possibly the microfibrillar-associated glycoprotein precursors of elastin (Cleary & Gibson, 1983).

In normal tissues neither junctions nor nexi are found between fibroblasts although they occur between cultured fibroblasts (Gabbiani & Rungger-Brandle, 1981). In this fibrous syncytium the presence of gap junctions is significant for they are considered as low resistance pathways (Pinto Da Silva & Gilula, 1972) through which cytoskeletal contact may be maintained and cellular messages transmitted (Beaulaton & Lockshin, 1982). Metabolic coupling is probably an important factor in producing an effective periosteal response to trauma and may account for the extension of the proliferative reaction seen at some distance from a site of injury (Tonna, 1966).

Small bundles, considered to be Sharpey fibres (Weinmann & Sicher, 1955), are clearly demonstrated by the picrosirius red stain and extend from the bone to pass between the cells of the cambial layer into the fibrous periosteum. With maturity these bundles become more evident, and the connection between bone and the periosteum is strengthened as the fibril diameters increase and the bundles enlarge. As this occurs, the effective level of mechanical stress needed to stimulate the cambial layer is raised.

Cellular degeneration within the periosteum occurs at two stages of development. Isolated cellular degeneration is seen initially during the early stages as the osteogenic layer differentiates. At this stage of development, prior to the establishment of an effective vascular supply, programmed cell death is commonly seen in many tissues (Beaulaton & Lockshin, 1982). In the later phases, as growth slows markedly and maturation proceeds, cell debris is seen in the region of the mid-zone as it gradually involutes and finally disappears. The elimination of unnecessary cellular components is characteristic of atrophy of a non-functional tissue. Disappearance of the mid-zone indicates a change in the functional status of the periosteum with age. With maturity, when the form of the bone is established and turnover is slowed, fewer progenitor cells are required. The metabolic demands of the tissue are considerably lessened and the mid-zone atrophies. The modulatory function, no longer as finely tuned as previously, appears to be taken over by the collagen bundles which can circumvent stress on the resting osteoblasts. The possible reappearance of the mid-zone in mature animals in association with conditions of fracture, increased stress or hormonal imbalance has not been fully investigated. There are several reports, which illustrate, without comment, thickening of the periosteum during bone repair (Tonna, 1966; Ashhurst, 1986).

In conclusion, the structure of the periosteum reflects the developmental stage of the

animal, providing remodelling during growth and stability at maturity. Interaction between the bone-forming surface and the overlying soft tissues is accomplished through the relatively loose connective tissue of the mid-zone. Its structure and composition is such that it can act as a buffer to protect the cellular periosteum from stress fluctuations within the physiological range so that only those of excessive duration and magnitude directly influence osteoblast function. With age and deletion of the mid-zone the fibrous periosteum appears to be anchored directly to the bone. The mid-zone has been overlooked in previous studies and this oversight has prevented an appreciation of its important role in bone remodelling.

This study serves as a benchmark for further investigations into the role of the periosteum in stress-induced bone remodelling.

SUMMARY

Female Sprague–Dawley rats from birth to 300 days were used to study the bone/soft tissue interrelationships of the 14th caudal vertebra with particular emphasis on the periosteum throughout growth, development and maturation.

The growth of the rats follows a sigmoid curve with three phases, a developmental, a rapid growth and a maturation phase. The width/length ratio of the bone and the thickness of the periosteum are closely concurrent, with a rapid decrease during the developmental phase and a levelling off during the rapid growth phase. SEM studies established that the caudal vertebra has symmetrical lateral sides and a pronounced concavity on the ventral surface where the main vascular plexus is located.

Morphological changes in the periosteum can be described as occurring in three layers and reflect the stages seen in general somatic growth. The inner cambial layer initially contains elongated but functional osteoblasts; these become cuboidal during the rapid growth phase and ultimately are flattened and quiescent. The mid-zone with its vessels, undifferentiated and mononuclear phagocytic cells also attains its maximum development in the rapid growth period and then gradually involutes. The fibrous periosteum consists of a syncytial arrangement of fibroblasts in a collagenous matrix which becomes increasingly dense although reduced in width. Sharpey fibre bundles connect the bone with the fibrous periosteum and these become thicker with age.

The mid-zone of the periosteum has not been described previously. Besides having a nutritive role and providing progenitor cells it is thought to act as a buffer modulating the interaction between bone and the covering soft tissues. With age and the deletion of the mid-zone a less sensitive periosteal response to stress can be expected.

This study was supported by a University of Melbourne Research Promotions Grant. We thank S. Ramm-Anderson and E. Allen for skilled technical assistance and C. Owen and S. Cleal for aid with the illustrations. We extend our gratitude to Professor E. Storey and Dr K. Ham for their valuable advice.

REFERENCES

ACHESON, R. M., MACINTYRE, M. N. & OLDHAM, E. (1959). Techniques in longitudinal studies of the skeletal development of the rat. *British Journal of Nutrition* **3**, 283–292.

ALTMAN, J. & SUDARSHAN, K. (1975). Postnatal development of locomotion in the laboratory rat. *Animal Behaviour* **23**, 896–920.

ASHHURST, D. E. (1986). The influence of mechanical conditions on the healing of experimental fractures in the rabbit: A microscopical study. *Philosophical Transactions of the Royal Society of London, B* **313**, 271–302.

BAIRD, I. L., WINBORN, W. B. & BOCKMAN, D. E. (1967). A technique of decalcification suited to electron microscopy of tissues closely associated with bone. *Anatomical Record* **159**, 281–290.

BARON, R., VIGNERY, A. & HOROWITZ, M. (1984). Lymphocytes, macrophages and the regulation of bone remodeling. In *Bone and Mineral Research Annual 2* (ed. W. A. Peck), pp. 175–243. Amsterdam: Elsevier.

BEAULATON, J. & LOCKSHIN, R. A. (1982). The relation of programmed cell death to development and reproduction: comparative studies and an attempt at classification. *International Review of Cytology* **79**, 215–235.

CHONG, D. A. & EVANS, C. A. (1982). Histologic study of the attachment of muscles to the rat mandible. *Archives of Oral Biology* **27**, 519–527.

CHONG, D. A., EVANS, C. A. & HEELEY, J. D. (1982). Morphology and maturation of the periosteum of the rat mandible. *Archives of Oral Biology* **27**, 777–785.

CLEARY, E. G. & GIBSON, M. A. (1983). Elastin-associated microfibrils and microfibrillar protein. *International Review of Connective Tissue Research* **10**, 97–209.

CRILLY, R. G. (1972). Longitudinal overgrowth of chicken radius. *Journal of Anatomy* **112**, 11–18.

FEIK, S. A. & STOREY, E. (1983). Remodelling of bone and bones: growth of normal and transplanted caudal vertebrae. *Journal of Anatomy* **136**, 1–14.

GABBIANI, G. & RUNGGER-BRANDLE, E. (1981). The fibroblast. In *Tissue Repair and Regeneration* (ed. L. E. Glynn), pp. 1–50. North Holland: Elsevier.

HAM, A. W. (1974). *Textbook of Histology*, 7th ed., Ch. 18. Philadelphia: Lippincott.

HAMILTON, W. J., BOYD, J. D. & MOSSMAN, H. W. (1962). *Human Embryology*. Baltimore: Williams & Wilkins.

HARKNESS, E. M. & TROTTER, W. D. (1978). Growth of transplants of rat humerus following circumferential division of the periosteum. *Journal of Anatomy* **126**, 275–289.

JUNQUEIRA, L. C. U., BIGNOLAS, G. & BRENTANI, R. R. (1979). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochemistry Journal* **11**, 447–455.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27**, 137A–138A.

LANYON, L. E. (1982). Mechanical function and bone remodeling. In *Bone in Clinical Orthopaedics* (ed. G. Sumner-Smith), pp. 273–304. Toronto: W. B. Saunders.

LUTFI, A. M. (1974). The role of cartilage in long bone growth: a reappraisal. *Journal of Anatomy* **117**, 413–417.

ORD, M. J. & SMITH, R. A. (1982). Correlation of mitochondrial form and function *in vivo*: microinjection of substrate and nucleotides. *Cell and Tissue Research* **227**, 129–137.

PINTO DA SILVA, P. & GILULA, N. B. (1972). Gap junctions in normal and transformed fibroblasts in culture. *Experimental Cell Research* **71**, 393–401.

POLLARD, A. W., FEIK, S. A. & STOREY, E. (1984). Remodelling of bone and bones: effects of translation and strain on transplants. *British Journal of Experimental Pathology* **65**, 655–670.

REYNOLDS, E. S. (1963). The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31–43.

STOREY, E. (1981). Form–function relationships of bone. In *Orthodontics: the State of the Art* (ed. H. G. Barrer), pp. 145–158. Philadelphia: University of Pennsylvania Press.

STOREY, E. & FEIK, S. A. (1982). Remodelling of bone and bones. Effects of altered mechanical stress on anlages. *British Journal of Experimental Pathology* **6**, 184–193.

STOREY, E. & FEIK, S. A. (1985). Remodelling of bone and bones: effects of altered mechanical stress on caudal vertebrae. *Journal of Anatomy* **140**, 37–48.

STOREY, E. & FEIK, S. A. (1986). Remodeling of bone and bones: effects of altered mechanical stress on the regeneration of transplanted bones. *Anatomical Record* **215**, 153–166.

TONNA, E. A. (1966). Response of the cellular phase of the skeleton to trauma. *Periodontics* **4**, 105–114.

TONNA, E. A. (1974). Electron microscopy of aging skeletal cells. III. The periosteum. *Laboratory Investigation* **31**, 609–632.

WARRELL, E. & TAYLOR, J. F. (1979). The role of periosteal tension in the growth of long bones. *Journal of Anatomy* **128**, 179–184.

WATTERS, W. B. & BUCK, R. C. (1971). An improved simple method of specimen preparation for replicas or scanning electron microscopy. *Journal of Microscopy* **94**, 185–187.

WEINMANN, J. P. & SICHER, H. (1955). *Bone and Bones*, 2nd ed., Ch. 2. St Louis, Missouri: Mosby.