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BONES

A STUDY OF THE DEVELOPMENT AND STRUCTURE OF THE VERTEBRATE SKELETON

BY

P. D. F. MURRAY, M.A., D.Sc.

With an Introduction by
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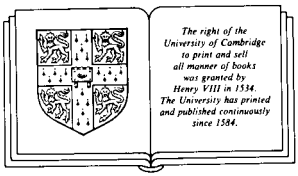

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CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore,
São Paulo, Delhi

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK

Published in the United States of America by
Cambridge University Press, New York

www.cambridge.org
Information on this title: www.cambridge.org/9780521315494

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First published 1936
Revised as a paperback with an introduction 1985
Re-issued in this digitally printed version 2009

A catalogue record for this publication is available from the British Library

Library of Congress Catalogue Card Number: 84–29392

ISBN 978-0-521-31549-4 paperback

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EQUALLY
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PREFACE

This book does not attempt to be comprehensive in its survey of the facts and theory of skeletal structure and development; that would require a book many times the size of this. I have merely tried to discuss in a brief space certain aspects of the development and structure of the skeleton, and in particular the question of the relation between the structures seen and the morphogenic factors intrinsic in the elements on the one hand, and those, mainly of a mechanical nature, which act upon it from without, on the other. In doing this, I have tried to present a fair picture of the present position of a controversial subject and to indicate, sometimes perhaps by daring to venture a little beyond the safe entrenchments of established fact, what seem the probable directions of future theoretical development and the lines of most profitable investigation. The main mass of the book is in the first four chapters, which deal with the embryonic and post-embryonic development and with the structure of the normal and modified bony skeleton considered in relation to the function it fulfils. In a brief fifth chapter cartilage is dealt with from the same point of view. The sixth is devoted to a consideration of the mechanisms by which mechanical forces find expression in the architecture of bone, and the seventh is a very brief summary.

Thanks are due to Dr H. B. Fell, Dr A. Glücksmann, Dr W. Jacobson, for a great deal of helpful advice and criticism, and for permission to mention their unpublished works, but particularly to Mr C. Mason, of the Cambridge Instrument Company, who devoted a great deal of time and trouble to assisting me with the mechanical passages, and to my wife,

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P. D. F. M.

September, 1936

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INTRODUCTION

Research in the development and structure of the skeleton since the publication of Murray's Bones

Introduction

It is half a century since Cambridge University Press published *Bones: A Study of the Development and Structure of the Vertebrate Skeleton* by P. D. F. Murray. At the time of its publication Murray, just 36 years old, was Smithson Research Fellow at the Strangeways Research Laboratories in Cambridge, England. Scion of a family whose accomplishments spanned scholarship, administration and politics (his uncle was Sir Gilbert Murray, the renowned classical scholar and Regius Professor of Greek at Oxford; his father, Sir Hubert, was for 32 years Lieutenant-Governor of Papua, and his grandfather, Sir Terence, was President of the Legislative Council of New South Wales, Australia), Murray won a University Medal upon graduation from Sydney University in 1922, proceeding to take a BSc at Oxford in 1924 and a DSc at Sydney in 1926, the latter based on the first of a life-long series of studies on the development of the skeleton. He pioneered the technique of grafting embryonic skeletal tissues onto the chorioallantoic membranes of host embryos as reported in three papers published in 1925 with J. S. (later Sir Julian) Huxley, papers published when Murray was just 25 years old. His interest in the relative roles of extrinsic and intrinsic factors in the acquisition of skeletal form developed early, as evidenced by an extensive review published with D. Selby in 1930. This topic formed a major theme of *Bones*. As Murray indicated in the Preface: 'I have tried to discuss . . . the question of the relation between the structures seen and the morphogenic factors intrinsic in the elements on the one hand, and those, mainly of a mechanical nature, which act upon it from without, on the

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other.' He was to review the topic again in the 1947 issue of the *Annual Review of Physiology*. Murray went on to publish on the origins of haematopoietic centres and fibrillation of the heart in the early embryo, on vitamin C deficiency in fracture repair and wound healing (his major work of the Second World War), and on the mechanical evocation of secondary cartilage from periosteal cells on avian membrane bones. *En passant*, he published one of the first integrative undergraduate textbooks on biology, a masterly fusion of zoology, botany, anatomy and physiology which was very widely used by both biology and medical students. A complete bibliography of Murray's papers may be found in the obituary published by the Australian Academy of Science (Rogers, 1968).

The publication of *Bones* marked a milestone in skeletal biology. No longer could the structure of the skeleton be viewed solely as a response to mechanical factors impinging upon it. Murray showed, in just 181 pages of succinct analysis and flowing prose, and using the experimental work which he and others had embarked upon, how the skeleton was shaped by a delicate balance of intrinsic and extrinsic factors. All three major 1937 reviews of the book (*Nature* **139**, 1036–7; *Quart. Rev. Biol.* **12**, 119; *J. Anat.* **71**, 582) emphasised this new paradigm. So convincing was Murray's case for intrinsic control of basic form that the reviewer in *Nature* commented that one 'cannot help being impressed by the inherent formative properties of the cells themselves. It is difficult to refrain from endowing them with potentialities that make further scientific investigation impossible' (p. 1037). Murray's insights into these 'inherent formative properties' led him to exactly the opposite conclusion. He ends the book with the positive affirmation of the experimental scientist that 'the real need is...for investigations of the mechanism which underlies the adaptations known to occur', and that such problems 'are

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probably not inaccessible to the ingenious mind and skilful technique of the biological experimenter' (p. 181). Indeed, they were not, as shown by Murray's subsequent work and by the explosion of experimentation since the publication of *Bones*.

Murray's book continues to be cited as a benchmark in the field. Its influence extends well beyond biology; it is cited in the primary literature of anthropology, anatomy, dentistry, engineering, medicine and veterinary science. In this brief introductory essay I want to present an overview of the progress which has been made in understanding what Murray called 'the obscure processes of skeletogenesis'. All that has happened since 1936 cannot possibly be covered. Rather, I will evaluate the major topics discussed by Murray and briefly summarise where we now stand.

The origin of skeletal cells

Murray began the book with a discussion of the development of the cartilage which forms the primary model for the long bones of the limbs, bones which form by endochondral ossification (mammals) and/or by sub-periosteal ossification (birds). He only made passing reference to the craniofacial skeleton and to the vertebrae (pp. 2, 27, 29, 175), for virtually nothing had been done on these regions of the skeleton. We now have data on all regions of the skeleton for a large number of vertebrates. Much of the data for many groups is still only descriptive, especially for fishes, reptiles and many mammals, but much other deals with underlying mechanisms. The Appendix gives an indication of the range of general works available. It is not exhaustive but was simply chosen by scanning the bookshelves of my office. For a further topically organised list of references see Ham and Cormack (1979).

Murray opens with a discussion of condensation of

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mesoderm – the first evidence of a future skeletal element. A condensation consists of preskeletal cells and occurs at the site of future skeletogenesis. Much is now known about the condensation process, both *in vivo* and *in vitro*. Three mechanisms are operative: division of cells *in situ*, aggregation or migration of cells towards the future skeletal site and suppression of cell death at the site (Hall, 1978, chap. 5; Bowen and Lockshin, 1981; Ede, 1983; Fyfe and Hall, 1983; Solursh, 1983). These local accumulations of cells form a skeleton themselves – what Grüneberg (1963) has called the membranous skeleton of the embryo (see also Ede, 1983).

Condensation places the mesenchymal cells sufficiently close together that they can begin to interact with one another. Such interactions are confined to cells at particular stages of differentiation (raising the possibility that they might be consequences not causes of such differentiation), and often involve extracellular matrix products such as collagen, or alterations in intracellular molecules such as cyclic AMP. Rounded cell shape is critical for such differentiative interactions (Abbott and Holtzer, 1966; Glowacki, Trepman and Folkman, 1983; Solursh, 1983), but we still have a long way to go in understanding how altered cell shape relates to the initiation of skeletogenesis. Evidence from analysis of mutants in which skeletal development is affected indicates that attaining a critical condensation size is often, but not always, necessary for the initiation of chondrogenesis or osteogenesis (Grüneberg, 1963; Grüneberg and Wickramaratne, 1974).

A major aspect of mesenchymal condensation which has come to light since Murray's book is the observation that skeletogenic mesenchymal cells migrate to their final site from another region of the embryo. Although these cells condense *in situ*, they do not arise *in situ*. Future vertebral cells (the sclerotome) move from the somites to take up positions adjacent

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to the spinal cord (Hall, 1977). Cells of the craniofacial skeleton arise in the neural crest, a ridge of cells along the dorsal surface of the neural tube; i.e. some of the skeleton arises not from *mesoderm* but from *ectoderm* – heresy in the 1930s but common parlance today. These neural crest cells leave the neural tube to undergo extensive migrations, first to create the craniofacial processes themselves and then to differentiate into the cartilage, bone and dentine of the chondro- and osteocranium, visceral arch skeleton and teeth (Noden, 1980, 1983; Le Douarin, 1982). Future craniofacial myogenic cells arise *in situ*. In the developing limb bud it is the future skeletal cells which arise *in situ* and the myogenic cells which arrive from outside, in this instance from the somatic mesoderm (Chevallier, Kieny and Mauger, 1977; Christ, Jacob and Jacob, 1977). The mesenchyme of the regions in which the primary skeleton and its musculature develops is therefore heterogeneous, although much of the experimental work of the 1960s and 1970s especially that on chondrogenesis and myogenesis *in vitro*, treated the mesenchyme as a homogeneous population of equivalent cells. Murray wrestled with this problem on the first page of his book. Are the cells in the condensations predetermined or are they indifferent with respect to their differentiative fate? Both their distinctive embryological origins and the interactions which occur between them argue for determination having occurred by the time that condensation takes place.

The shape of that initial condensation is not randomly generated but is established with remarkable precision well before any cartilaginous or bony matrix has been deposited, as elegantly demonstrated by Noden (1983) and by Hinchliffe (1977). (See Hinchliffe and Johnson (1980) for a thorough discussion.) Within that condensation the shape of the future skeletal rudiment is foreshadowed (discussed on pp. 6–10 of

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Bones). Is that shape generated by positional effects as postulated by Wolpert (1978), by mechanical contractile forces associated with cell movement (Oster, Murray and Harris, 1983), by morphogens (Turing, 1952), or by differential growth (Wolpert and Hornbruch, 1981)? Although these mechanisms vary greatly, they have as a common denominator the concept of intrinsic control, independent of adjacent tissues and of the biomechanical factors which mould so much of the subsequent shape of the skeleton (see pp. *xxiv–xxxii*).

Thus, the skeleton arises from cells which often have to migrate to the site of skeletogenesis, there to form condensations, a process which allows similarly determined cells to interact with one another. Such future skeletogenic cells may be distinguished from cells outside the membranous skeleton by this determination for chondrogenesis or osteogenesis. As Murray points out (pp. 3–5), cartilage- and bone-forming cells are not separate cell lineages. He cites the classic work of Fell (1933) on formation of cartilage from endosteal cells *in vitro*. Murray himself went on to document the formation of cartilage from otherwise osteogenic periosteal cells of membrane bones (Murray, 1957, 1963; Murray and Smiles, 1965). Such secondary cartilage is now routinely recognised in histology texts as a major class of cartilage and has a book devoted to it (Beresford, 1981). These periosteal progenitor (stem) cells are bipotential, forming cartilage when subjected to mechanical forces, but otherwise forming bone. The existence of secondary cartilage is but one example from the many studies which have firmly established the concept of the skeletogenic or osteochondrogenic stem cell, and which have shown that cartilage and bone (and often connective tissue as well) are an interrelated family of tissues which arise by modulation from such stem cells (Bassett and Ruedi, 1966; Hall, 1970, 1978; Owen, 1970; Beresford, 1981).

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Cells *outside* the condensations may also have skeletogenic potential. Murray (pp. 3–4, 151–2) discusses the classic study of Huggins (1931) which demonstrated that urinary bladder epithelium induces bone from connective tissue cells in some tissues but not in others. Urist, Dowell and Hay (1968) have confirmed this spatial heterogeneity of responsive cells and the pioneering work of Friedenstein in the USSR has identified two classes of osteoprogenitor cells in the adult, but outside the skeleton, one which is already determined for osteogenesis (his DOPC, determined osteogenic precursor cells) and one which has to be induced before it will deposit bone (his IOPC, inducible osteogenic precursor cells: Friedenstein, 1973, 1976; Owen, 1978). As with cells within condensations, such precursors can also form fibrous tissue, i.e. they are bipotential, a phenomenon especially well illustrated by intratendinous ossification (see Murray, pp. 114–17 and 153–4; Wong and Buck, 1972; McClure, 1983). Proliferating epithelia are potent inducers of the subset of adult cells capable of responding to them (Wlodarski, 1969; Anderson, 1976; Hall 1983*a*). Epithelia also play a critical role in evoking chondro- and osteogenesis *within* condensations (Hall, 1983*a*, *b*), highlighting the commonality of epigenetic interactions in embryonic and post-foetal skeletogenesis. Other potent inducers of ectopic skeletogenesis have been discovered since 1936, notably bone morphogenetic protein, a glycoprotein of molecular weight 17500 resident within bone matrix (Urist, 1980, 1983). This may or may not be the same factor as that isolated by Reddi (1983), a protégé of Huggins who started the field 55 years ago. There are two common denominators which emerge when considering intra- and extraskeletal osteogenesis and chondrogenesis: the existence of competent cells and the presence of a triggering mechanism to activate those cells.

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Intra- and extraskeletal regulation of osteogenesis also act together in the osteoclast. Murray had very little to say about these bone-resorbing cells (pp. 162–6). Without doubt it is our changing view of the osteoclast which has most revolutionised the study of the skeleton. It was dogma for a century that osteoclasts and osteoblasts arose from progenitor cells *within* the skeleton, indeed that they were modulations of the *same* progenitor cells. The treatments underlying hormonal control of bone growth, remodelling and decay, and the treatment of metabolic bone diseases, started with this premise. Over the past ten years our conception of osteoclast origins and activation has changed dramatically, for it is now known that the osteoclast arises *outside* the skeleton, from precursors circulating within the blood stream, that these cells have to migrate to the skeleton and that their resorptive action, long known to be correlated with cycles of deposition of bone, is so coupled because osteoblasts release factors which activate quiescent osteoclasts or their precursors (Owen, 1970, 1978; Hall, 1975; Ham and Cormack, 1979; Rodan and Martin, 1981; Mundy *et al.*, 1982; Malone *et al.*, 1982; Parfitt, 1984). Osteoblasts and osteoclasts are separate cell lines, united not by commonality of origin, but by functional interaction. This interaction is reflected in the current *in vitro* and grafting approach to osteoclast function, which is to co-culture or graft osteoclasts with bone as a matrix to be resorbed and with osteoblasts to provide the diffusible activating factors (Krukowski and Kahn, 1982; Chambers, 1982; Holtrop, Cox and Glowacki, 1982; Zambonin Zallone, Tetti and Primavera, 1984). Indeed, heterogeneity of osteoclast populations is beginning to emerge, as in the recent studies of Liu and Baylink (1984) and of Marks (1983). Is such heterogeneity based on separate races of osteoclasts or on specificity of activation? The documentation of such heterogeneity – fibroblasts with skeletogenic

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potential versus those without, site-specific populations or activation of osteoclasts, or the non-equivalence between a chondrocyte which goes to make up a wrist element and one which makes up a digit or humerus – is a major future task in our continuing search for the origins of skeletal cells and the control of their differentiation.

Morphogenesis of cartilage

A further major contribution made by Murray in *Bones* was the marshalling of evidence and arguments to show that the basic form of long bones – the relation of length to width, cross-sectional shape, basic longitudinal curvature, shape of major condyles – was not an adaptive response to mechanical influences, but rather was intrinsically programmed within the cells of the cartilaginous primordium (pp. 3–42).

From the mid 1920s on, cartilaginous rudiments were being organ cultured or grafted in isolation from muscular, nervous and/or vascular influences, and in isolation from influences of the cartilaginous rudiment with which they normally articulated. Such cultured and grafted cartilages formed skeletal elements of surprisingly normal morphology. Murray especially emphasised the normality of the shape of the shaft (pp. 4–6), articular structures (pp. 13–17 and figs. 2 and 3) and the major grooves and tuberosities which would, in real life, have accommodated ligaments and muscles (pp. 18–19). Dame Honor Fell, long-time Director of the Strangeways Research Laboratories where much of this early work was done, has reviewed the organ culture experiments (Fell, 1969, 1976); Felts (1961) has reviewed the grafting experiments; and recently Thorogood (1983) has provided a comprehensive overview of cartilage morphogenesis, including an evaluation of Murray's contributions, and discussions of the relationship between

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intrinsic and extrinsic factors in cartilage morphogenesis and between morphogenesis and growth. Thorogood identifies seven factors which impinge upon cartilage morphogenesis, viz., orientation of cells within the condensation, cell division, cell hyperplasia, secretion of extracellular matrix, growth of the cartilage, physical constraint of adjacent tissues and external mechanical stimuli. The fundamental shape of the cartilage is influenced by all of these factors but the basic intrinsic control lies within the condensation and particularly in the orientation of the cells within the condensation (Ede, 1971; Ede, Hinchliffe and Balls, 1977; Trelstad, 1977; Ede and Wilby, 1981; Thorogood, 1983). Differential adhesion of cells within the condensation plays a major role in that orientation (Ede, 1983). Somewhat later, the constraining influence of the perichondrium also comes into play (Wolpert, 1981).

That the behaviour of prechondrogenic cells within condensations is both intrinsic and cartilage-type specific is dramatically illustrated by the experiments of Weiss and Moscona (1958) where limb or scleral chondrogenic mesenchyme, upon dissociation into single cells and subsequent cell culture, forms rods or sheets of cartilage, respectively – forms which are equivalent to the shafts of long-bone primordia or to sheets of scleral cartilage. Such specific reassociation requires cell–cell interaction, for diluting the cell cultures with fibroblasts destroys the morphogenetic specificity of the reaggregate (Archer, Rooney and Wolpert, 1983). It is the surfaces and superficial extracellular matrices of the cells in the condensation that we will need to study if we are to understand the morphogenetic individuality of determined skeletogenic cells. Intrinsic control of skeletal growth can also be traced back to the condensation (Hall, 1982; Hinchliffe and Johnson, 1983), as is especially well shown when anlage of skeletal elements are grafted into hosts whose skeletons have different growth rates,

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final sizes or shapes (Twitty and Schwind, 1931; Iten and Murphy, 1980). More such studies are required.

Chondrones

Murray described chondrones as a basic structural unit within cartilage (pp. 136–48); each chondrone was thought to consist of a group of several chondrocytes encircled by a common fibrous network, with groups of chondrocytes similarly united by fibres until the entire cartilage came to constitute a linked set of chondrones (fig. 41, p. 140). The existence of such structural units has not stood the test of modern-day transmission and scanning electron microscopy (Stockwell, 1979; Boyde and Jones, 1983), so that chondrones are rarely mentioned these days. When they are, it is in relation to degenerative changes of cartilage in ageing or in pathology (Sokoloff, 1969; Stockwell, 1979). Unlike the osteon, which is the structural unit of bone, the chondrone has not been verified as a basic structural unit, despite our very extensive knowledge of the structural basis of cartilaginous extracellular matrix – cartilage-specific collagen and proteoglycan, glycosaminoglycan structure and attachment to collagen, and aggregation of matrix products (Lash and Vasan, 1983; Mayne and von der Mark, 1983).

Although chondrones have not been confirmed as the structural unit of cartilaginous extracellular matrix, considerable heterogeneity of the matrix does exist, especially when the territorial (pericellular) extracellular matrix around each chondrocyte is compared with the extraterritorial matrix between chondrocytes (Eisenstein, Sorgente and Kuettner, 1971, 1973; Kashiwa, Luchtel and Park, 1975; Farnum and Wilsman, 1983). Improved fixation and the use of ruthenium complexes has demonstrated the association of matrix

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components such as collagen and proteoglycan and the specific contact of pericellular proteoglycans with the chondrocyte plasmalemma (Shepard and Mitchell, 1977; Hunziker, Herrmann and Schenk, 1983; Hunziker and Schenk, 1984). These technical advances, along with the use of immuno-electron microscopy of specific matrix products (proteoglycans, collagen, link proteins) and electron spectroscopic imaging of elements such as calcium, phosphorus and sulphur (Poole *et al.*, 1982; Arsenault and Ottensmeyer, 1983), should enable us to break the code of cartilage's structural organisation within the near future.

The formation of joints

Murray dealt with the problem of the origin of the articulations which unite skeletal elements, both the freely movable synovial joints between long bones (pp. 7–26, 81–9) and sutures between the dermal bones of the cranium (pp. 65–7). He was concerned with the factors which determined where an articulation would develop, with initiation of the joint and with what moulds its final shape, and used evidence from pseudarthroses to show that joints could form in other than their normal sites and that both the initiation and the morphogenesis of such false joints were under mechanical control. A similar mechanism has been invoked by evolutionary biologists to explain the origin of new joints during evolution (Bock, 1960; Frazzetta, 1970). Murray showed that, in contrast, *primary* joints developed independently of any such mechanical stimulus, although he did not rule out 'developmental interactions between the skeletal segments' (p. 13) as playing a role. His chorioallantoic grafting experiments showed how normal the articular surfaces of such isolated elements could be (see his fig. 3, p. 15). Subsequent work has substantiated his views, a

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reasonable summary being that initiation of joints is independent of function while final differentiation and maintenance of the form of a joint are dependent upon extrinsic mechanical factors (see the papers in Sokoloff, 1978, 1980). Formation of the joint often only proceeds as far as initial cavitation, function being required to complete both cavitation and further differentiation, as well as to maintain the joint (Drachman and Coulombre, 1962; Drachman and Sokoloff, 1966; Beckham, Dimond and Greenlee, 1977;Mitrovic, 1982). Forces generated by growth of the adjacent skeletal element are not sufficient (Holder, 1977), although cell alignment is important, as it is for cartilage morphogenesis (Henrikson and Cohen, 1965). In a paper published after Murray's death (Murray and Drachman, 1969), paralysis was used to demonstrate how joints that are already formed ankylose when deprived of mechanical stimulation (see also Sullivan, 1966, 1974).

The development of sutures in the cranium has been a particularly difficult area of investigation. Are sutures predetermined boundaries or do they merely develop where two bones happen to meet? (p. 65). Along with this problem goes that of the amount of bone growth which occurs at the sutural margin. Classically, osteogenesis at sutures was considered to provide a minimal contribution to growth of the skull (Brash, 1934). It is now known that, although growth can be quite different and specific, both on opposite faces of the bone and on opposite margins of the suture, such growth is adaptive and not a driving force in skull growth (Young, 1962; Moore and Lavelle, 1974; Moore, 1981). Furthermore, bone can be deposited where a suture would normally develop when sutural tissue is extirpated or when sutural fusion occurs – i.e. presumptive sutural soft tissue has osteogenic potential (Moss, 1959; Ritsila, Alhopuro and Ranta, 1973; and Hall, 1978, for a review).

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The structure of sutures is especially well adapted to resist tension. Local areas of pressure or compression are met by the ability of cells in the suture to become chondrogenic, thus resisting and adapting to the local needs (Beresford, 1981). Again, intrinsic skeletogenic potential, expressed under extrinsic control, is paramount.

The trajectory theory and bone structure

Bone as an organ serves two primary functions, for it provides both a structural support and a reserve of calcium and phosphorus. As a *tissue*, bone is adapted to modify its behaviour and/or structure to serve these two functions, in the former case by growth or remodelling in response to mechanical stresses and in the latter by responding to altered serum levels of calcium and phosphorus and to changed hormonal levels.

A considerable portion of *Bones* is devoted to the trabecular structure of bone and to bone structure as adapted to and accurately mirroring the stress to which it is subjected (pp. 94–135, 155–74). The trajectory theory of Wolff posits that the trabeculae of cancellous bone follow the lines of trajectories in a homogeneous body of the same form as the bone and stressed in the same way. They are thus regarded as actually materialised trajectories (pp. 99–100 and see figs. 29–30, pp. 108–9). Murray reviewed several lines of evidence – the structure of the trabeculae in the long bones of marine mammals such as the dolphin (fig. 23, p. 100), fibre orientation, osteon arrangement, muscle action, the structure of the bone which forms in tendons, and the absence of tension trajectories, the temporal correlation between orientation of trabeculae and onset of function – and concluded 'that bony structures are therefore in a general way mechanically adaptive, but that the trajectorial theory in its rigid form demands a degree of perfection

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in this adaptation which does not exist in fact, because the mechanical stresses and strains of function are not the only factors which influence the development of bony architectures' (p. 135).

What is the current status of the trajectory theory and do we now know how bones adapt their structure to meet an altered mechanical environment? Although specialists such as orthopaedic and oral surgeons and orthodontists spend their working lives manipulating the skeleton in conformity with the trajectory theory, little has been explicitly done on it over the past several decades. Is this because, like gravity which it resists, it is no longer a theory but a truism, to be assumed and therefore left unstated, or is it because it is a theory which is no longer accepted, bone having to conform to various extrinsic factors and not just to stress? Examination of numerous recent texts reveals either acceptance of only a general moulding of form along trajectorial lines (Urist, 1980, p. 114; Owen, Goodfellow and Bullough, 1980, p. 68), arguments that a strict trajectory model must be incorrect (Ashton and Holmes, 1981, pp. 147–50), or, in most texts, no mention of the theory at all.

Perhaps the textbooks are lagging behind research in the field. Several studies in the primary literature have examined trabecular structure in the light of the trajectory theory. Currey (1968) provides a very thoughtful review of some of these studies.

Felts and Spurrell (1965, 1966) examined whale humeri but could not provide strong support for the theory, structure being related more to the physiological adaptations of life in cold water. Dempster (1967) expressed similar caution from his studies on the structure of the human skull, emphasising the weakness of bone in tension, and that, at best, trabeculae follow lines of maximum stress. Enlow (1968) emphasised the importance of considering the structure and response of bone

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to stress in the context of its soft tissue matrix (what Moss (1962) termed the functional matrix), a matrix which may dramatically modify the response of the bone when compared with how an isolated skeletal element might respond. This was also emphasised by Lanyon (1974) in his strain gauge analyses. The point is nicely illustrated by the biomechanics of the oral region of the dog (Scapino, 1967). In the upper jaw it is the anterior teeth on the maxilla which absorb much of the force generated in biting, while in the mandible the palatal mucosa develops a specialised, highly fibrous connective tissue which absorbs these forces; these two quite different adaptations to resist mechanical stress both result in a bony architecture considerably modified from that which would be predicted if the forces acted directly upon the bone of the jaws. Similar conclusions have been drawn from analysis of the avian bill (Bock, 1966). Nor does trabecular architecture correlate with mechanical forces in very small mammals, such as bats and shrews. Wolff's law does not apply, and instead intrinsic tissue strength moulds bony architecture in such animals (Dawson, 1980, and see also Hanken, 1982, for small salamanders). Similarly, in the development and structure of the vertebrae of bony fishes, the fundamental biconid shape is not stress-related but rather is a consequence of the vertebrae developing from mesenchyme which lies beside a notochord alternately constricted and dilated along the embryonic axis. Only the subsequently formed trabeculae of the spongy bone are deposited along lines dictated by mechanical stresses (Laerm, 1976). The final structure of the bones reflects both major influences on their development. On the other hand the *sequence* of ossification is functionally related, the first-formed bones being those associated with resisting the mechanical stresses of feeding and respiration (Weisel, 1967).

The trajectory theory presents idealised structures formed

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in response to pure forces by an isolated bone whose only function is to resist those mechanical forces. Theoretical approaches which treat bones as idealised, isolated units, as in the application of catastrophe theory (Sinha, 1981), simply fall short of reality. A particular bone's response to altered mechanical stress might be compromised by the simultaneous response of the attached muscle or connective tissue (muscles may insert onto resorptive surfaces in which case muscular activity will stimulate bone resorption and not deposition – a factor not usually taken into account: Hoyte and Enlow, 1966; Enlow, 1975), by altered blood flow, by associated mineral requirements, etc. Just as in the embryonic condensations, so in the adult we cannot divorce the skeleton from its environment. Murray foresaw this interactive view and elegantly expressed it in his concluding chapter (pp. 177–80): 'Every bony structure is a compromise and no compromise is perfect' (p. 179). 'It is the question of the kind of functional stimulus which is effective and the manner of its action that remain as the most obscure problem in skeletogenesis' (pp. 179–80). Some of these stimuli will be discussed in the next section.

Growth of cartilage and bone

Murray considered several aspects of growth, including determination of the lengths of the skeletal elements, and concluded that 'undiscovered factors' such as those controlling rate of spread of chondrogenesis and relative position of chondrogenic centres must be important (p. 25). Given that the chondrogenic cells have to migrate to their final sites (see p. *xv*), relative position is determined by components of extracellular matrices (collagen, fibronectin, hyaluronate, etc.) which control migration (Newgreen, 1982*a*, *b*, 1984), and by

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the epigenetic factors which signal that migration should cease, the primary candidates being epithelia or nerves (Moss, 1972*a*; Hall, 1978; Horder, 1978; Lumsden, 1979). The spread of chondrogenesis is a function of the rate at which cells mature and deposit extracellular matrix-processes which are primarily controlled by hormones and by the physical constraint of the perichondrium (Wolpert, 1981; Hinchliffe and Johnson, 1983; Thorogood, 1983). Growth of cartilage is under intrinsic control by factors such as the number of stem cells in a condensation and the proportion of those cells capable of mitosis; the amount and rate of hypertrophy of chondrocytes; and the amount and rate of synthesis, secretion and degradation of extracellular matrix (Moss, 1972*b*; Hall, 1978; Hinchliffe and Johnson, 1983). The latter two (hypertrophy and secretion of extracellular matrix) are much more important than cell division, a finding that would perhaps not have been predicted but which is now very well documented (Biggers and Gwatkin, 1964; Hall, 1978; Thorogood, 1983). A particularly well-studied example is the development and differential growth of the tibia and fibula in the embryonic chick, where the original notion of competitive interaction for limited mesenchyme to form the initial condensations has been augmented by studies which focus on the rate of hypertrophy and matrix synthesis; these studies have been reviewed by Hinchliffe and Johnson (1983) and by Hall (1984). Hypertrophy and cell division are separately regulated. Thyroid hormone stimulates chondrocyte hypertrophy without affecting cell division (Burch and Lebovitz, 1982). Freezing at −80 °C suppresses growth by inhibiting hypertrophy without affecting cell division (Biggers, 1957).

There is considerable evidence for feedback from extracellular matrix to the chondrocytes as a factor in cartilage growth, both *in vivo* and *in vitro* (reviewed by Hall, 1978). The

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various genes known to produce achondroplasia (chondrodystrophy) – genes such as *cartilage anomaly* (*can*), *achondroplasia* (*cn*), *chondrodysplasia* (*cho*), *brachymorphic* (*bm*) and *stumpy* (*stm*) in the mouse, *achondroplasia* (*ac*) in the rabbit, and *nanomelia* (*nm*) in the chick – all produce a similar growth inhibition and syndrome but act on quite different processes affecting growth, viz., protein synthesis (*can*), chondrocyte maturation (*cn*), glycosaminoglycan synthesis (*cho*), aggregation of glycosaminoglycans (*bm*), failure of chondroblasts to separate (*stm*), glucose metabolism (*ac*), or suppression of synthesis of cartilage-specific proteoglycan (*nm*) (Hall, 1978). These mutants provide a powerful example of the multiplicity of intrinsic controls over cartilage growth.

Hormones, vitamins and growth factors produced by cartilages themselves (cartilage-derived growth factor, chondrocyte growth factor, cartilage-derived factor) have profound effects on the growth of cartilage (Elmer, 1983; Ornoy and Zusman, 1983; Silbermann, 1983).

Murray also considered the growth of the bone which replaces the cartilaginous model during endochondral ossification, both the increase in thickness and length (pp. 70–80) and the excess deposition which occurs on the concave surface of a curved bone (pp. 31–41 and see figs. 5–8, pp. 32–4). He interpreted growth in length and width as a response to functional demand, i.e. although growth of the cartilaginous model is largely under intrinsic control, growth of the bone which replaces it is largely under extrinsic control. This conclusion has been amply confirmed by subsequent studies (see reviews by Moffett (1972), Goss (1978), Dixon and Sarnat (1982) and Hinchliffe and Johnson (1983), the last providing a systematic survey of the various groups of vertebrates). This extrinsic control is especially evident in the growth of the chondro- and osteocranium, where the sense organs and brain

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provide a functional matrix which regulates the rate of cartilage or bone growth. Skeleton, muscle, tendon and ligament act as a functional unit in controlling growth, a point emphasised by Murray (p. 91) and by many later studies (see Moss, 1972*a*; Moffett, 1972; Moore and Lavelle, 1974; Moore, 1981, for discussions). Muscular control is classically illustrated by (*a*) the cessation of growth that is seen in mammalian mandibular processes such as the condylar or coronoid process when specific muscles are ablated or congenitally absent, or the increased growth which accompanies augmentation of muscle mass (Moore, 1965); (*b*) the effect of genes such as *muscular dysgenesis* which suppresses muscle development and therefore inhibits skeletal growth (Pai, 1965*a*, *b*; Herring and Lakars, 1981); or (*c*) the effect of embryonic paralysis, prolonged bedrest or weightlessness on skeletal growth. The phenomenon of 'catch-up growth' further illustrates this extrinsic or epigenetic control (Williams, 1981). Extrinsic control of bone curvature has been elegantly demonstrated by Lanyon (1980) using unilateral sciatic neurectomy of rats. As he points out the altered tibial growth has to both accommodate altered muscular activity and optimise the new functional strains to which the tibia is exposed. Similar adaptations occur in bipedal rats (Riesenfeld, 1966).

Murray paid some attention to the differing effects of intermittent and constant pressure on bone growth (pp. 156ff). Constant pressure results in atrophy while release from that pressure or alternating pressure and lack of pressure favours growth (pp. 156, 163). It is now known that the response of various cell types (myoblasts, fibroblasts, periosteal progenitor cells) to cyclic mechanical stimulation is increased DNA synthesis and cell division (Leung, Glagov and Mathews, 1976; 1977; Curtis and Seehar, 1978; Hall, 1979). The forces to which most skeletal elements are exposed derive from respiration,

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blood flow or locomotion, all of which produce cycles, often of very short duration, of alternating tension and compression (Rodbard, 1970; Liskova and Hert, 1971; Lanyon, 1972; Lanyon *et al.*, 1975; Goodship, Lanyon and McFie, 1979). Only some models take this intermittency of mechanical stress into account (Gjelsvik, 1973*a*), but these are the physiological stresses to which cartilage and bone respond. Weightlessness under zero gravity in space shows how different skeletal cells are adapted to respond differently to the same stimulus, for weightlessness slows bone formation but not resorption (Jee *et al.*, 1983), and weight-bearing bones are at greater risk than are non-weight-bearing bones (Simmons *et al.*, 1983). It is not only growth which is regulated by such stimuli. The pathway of cell differentiation can also be selected in response to the type of mechanical stimulus. Periosteal progenitor cells on membrane bones respond to intermittent stresses by forming secondary cartilage, i.e. by differentiating as chondroblasts, and to constant pressure by forming bone, i.e. by differentiating as osteoblasts (Murray, 1963; Hall, 1968, 1979). Chondrocytes, rather than fat cells (adipocytes) or connective tissue cells (fibroblasts), form in mesenchyme or the vascular system when these tissues are exposed to intermittent mechanical stress (Rodbard, 1970; Yamada *et al.*, 1977; Hadjiisky *et al.*, 1979; Arbustini, Jones and Ferrans, 1983). Chondrogenesis is not initiated when constant pressure is applied to bone (Glücksmann, 1938; Hall, 1968). In this we come full circle in Murray's book, for here we have determined cells responding to extrinsic signals to initiate or promote skeletogenesis.

The critical question now is not whether skeletal growth and structure are controlled both intrinsically and extrinsically, for *Bones* and the last half century have answered that in the affirmative, but how the two levels are coordinated to effect

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skeletogenesis. Mechanical effects on cartilage and bone may be mediated by altered electrical activity – for the skeleton is piezoelectric and both cartilage and bone respond to changes in electrical activity (Currey, 1968; Gjelsvik, 1973*a*, *b*; Brighton, Black and Pollack, 1979; Hall, 1983*c*) – or by changes in calcium and cyclic AMP levels (Bourret and Rodan, 1976; discussed in Hall, 1978). Mechanisms of action of hormones as gene regulators and of vitamins acting via the cell surface are beginning to be understood (Silbermann, 1983; Ornoy and Zusman, 1983). Many growth factors which act specifically on cartilage or on bone are now known (Elmer, 1983; Mohan *et al.*, 1984). We need more understanding of these factors, but the fundamental levels of control still elude us. What does determine the size and shape of a condensation, induce chondrocyte hypertrophy, attract osteoclast precursors to bone, set the balance between mechanical needs and ion reserve as elaborated in bone structure, determine the width of a suture, establish differential growth rates, produce a rod of cartilage and not a sheet? What is the fundamental structural unit of cartilage? As Murray said in concluding *Bones*, these are 'problems whose answers would throw a flood of light on the obscure processes of skeletogenesis'.

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reasonable summary being that initiation of joints is independent of function while final differentiation and maintenance of the form of a joint are dependent upon extrinsic mechanical factors (see the papers in Sokoloff, 1978, 1980). Formation of the joint often only proceeds as far as initial cavitation, function being required to complete both cavitation and further differentiation, as well as to maintain the joint (Drachman and Coulombre, 1962; Drachman and Sokoloff, 1966; Beckham, Dimond and Greenlee, 1977;Mitrovic, 1982). Forces generated by growth of the adjacent skeletal element are not sufficient (Holder, 1977), although cell alignment is important, as it is for cartilage morphogenesis (Henrikson and Cohen, 1965). In a paper published after Murray's death (Murray and Drachman, 1969), paralysis was used to demonstrate how joints that are already formed ankylose when deprived of mechanical stimulation (see also Sullivan, 1966, 1974).

The development of sutures in the cranium has been a particularly difficult area of investigation. Are sutures predetermined boundaries or do they merely develop where two bones happen to meet? (p. 65). Along with this problem goes that of the amount of bone growth which occurs at the sutural margin. Classically, osteogenesis at sutures was considered to provide a minimal contribution to growth of the skull (Brash, 1934). It is now known that, although growth can be quite different and specific, both on opposite faces of the bone and on opposite margins of the suture, such growth is adaptive and not a driving force in skull growth (Young, 1962; Moore and Lavelle, 1974; Moore, 1981). Furthermore, bone can be deposited where a suture would normally develop when sutural tissue is extirpated or when sutural fusion occurs – i.e. presumptive sutural soft tissue has osteogenic potential (Moss, 1959; Ritsila, Alhopuro and Ranta, 1973; and Hall, 1978, for a review).

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