Vascular Architecture of the Mandibular Processes in Hamster and Rat

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An investigation of the vasculature of the mandible and its relationships with growth, development, and postsurgical remodeling.

KEY WORDS: • BONE REMODELING • CORONOID PROCESS • • MANDIBLE • VASCULATURE •

Anatomists, physical anthropologists, orthodontists, and oral, plastic, and orthopedic surgeons maintain abiding interests in the relationship between bone form and muscle function (WASHBURN 1946A, WASHBURN 1946B, AVIS 1959, RAY 1963, ENLOW 1965, ENLOW 1968, HOYTE AND ENLOW, 1966, BASSETT 1972, MOSS AND MOSS-SALENTIJN 1978, BURKE AND MCNAMARA 1979, MULLIKEN AND GLOWACKI 1980, ROBERTS 1981).

Wolff's law explains many facets of this complex muscle-bone interaction. As presently interpreted, Wolff's law states simply that the form and internal architecture of bones develop as adaptations to the sum of the applied biomechanical stresses. This concept is most useful in explaining changes in adult bone form and structure; however, this does not fully explain the genetic contributions that direct the origin and/or growth of the individual bones or bony processes, or the resizing and reshaping of bones as they achieve adult form during growth and development.

The basic problem is that the mechanisms controlling bone development and growth are still not fully understood. Available knowledge is based on criteria that describe *what* has happened, not *how* the events described happen.

Growth-controlling feedback mechanisms are apparently operating at the systemic, tissue, cellular and molecular levels. While there are research data that focus on each level, our information concerning such feedback mechanisms is very limited.

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An adequate vascular system (blood, blood vessels and the accompanying perivascular mesenchymal tissue) may be a local controlling factor essential for optimum bone growth and development. Indeed, an adequate microvasculature may be the common denominator for bone growth and development as well as the bone changes that occur during adult life.

For example, the microcirculatory system is critical during the formation of a bone that is preformed in cartilage (TRUETA AND MORGAN 1960). During this process, new capillaries invade the areas of calcified cartilage and the cells of the undifferentiated perivascular mesenchyme are presumed to differentiate into the cells of the bone-forming membranes, the periosteum and the endosteum.

These membranes then deposit the initial osteoid on the calcified cartilage spicules, and soon this primitive bone tissue is calcified. Thus, bone membranes are the actual agents that implement the continual resizing and reshaping of bones as they grow differentially in response to functional forces during bone remodeling through differential apposition and resorption.

These observations suggest also that blood, blood vessels and the perivascular mesenchyme are all vital to bone membrane growth and function.

The reshaping of bones by remodeling is expected by clinicians who surgically alter the spatial relationships of bone segments and/or muscles. Bone grafts are often used in the repair and reconstruction of the craniofacial region.

Three mechanisms are proposed to explain the success of bone grafts.

First, fresh autogenous grafts retain living cells (presumed to be supplied with new blood vessels) that participate in the osteogenic response in and around the graft (RAY 1963, BASSETT 1972, HAM AND GORDON 1952).

Second, the bone graft acts as a scaffold for the invasion of new blood vessels, followed by the differentiation of new membranes, the resorption of the graft and the later deposition of new bone tissue (THOROGOOD AND GRAY 1975).

Third, demineralized or devitalized bone grafts provide a trellis without living cells; therefore osteogenesis is induced by causing host cells to migrate, proliferate and differentiate into bone-forming membranes (RAY AND SABET 1963).

Fresh autogenous bone grafts appear to be the optimal grafting material (MULLIKEN AND GLOWACKI 1980), although undemineralized and demineralized bone grafts and hydroxyapatite compounds are satisfactory. The final shape of the bone graft is probably dictated by the sum of the biomechanical stresses and/or by the differential growth of new bone membranes.

Just how biomechanical stress is translated into genomic-directed bone remodeling remains unclear; however, ROBERTS (1981) divides bone remodeling into three major categories:

- Turnover in response to the accumulation of microfractures
- Reorientation of bone mass to optimally resist stress (Wolff's law)
- Net change in bone volume related to functional load.

Bone grafts activate or induce bone remodeling (resorption and apposition), and the process probably requires the activation of specific segments of the genome so that the new bone membranes can adapt the new bone tissue to the functional stresses. Functioning muscles and the new vasculature apparently play important roles in the success of these procedures.

BELL (1976) showed that monkeys subjected to pedicled vertical ramus osteotomies revealed no disruption of the circulation of the proximal segment, early

osseous union, and minimal osteonecrosis and vascular necrosis.

These observations contrasted strongly with those in animals that received non-pedicled vertical ramus osteotomies. The latter group had disrupted circulation in the proximal segment, delayed osseous union, and severe osteonecrosis. These data show the importance of an adequate vasculature.

If the function and microcirculatory systems of muscle have important roles in mandibular process osteogenesis and bone remodeling, it should be possible to design experiments to observe the interaction between the vascular systems of bone and muscle. Indeed, data in the literature indicate that the shape and size of mandibular angular processes (WASHBURN 1946B), coronoid processes (AVIS 1959, WASHBURN 1947) and condylar necks (BURKE AND MCNAMARA 1979) are strongly influenced by the functional stresses imposed by the attached muscles.

WASHBURN (1946B) showed experimentally that the embryonic angular processes will not develop without a functioning pterygo-masseteric muscle complex. Similarly, the research of AVIS (1959) demonstrated that a functioning temporal muscle was required for the embryonic development of the coronoid process.

The above experiments were based on the early ablation of specific muscle tissue. Critics quickly pointed out that the resulting abortive growth of the mandibular, angular and coronoid processes was caused by the disruption of the microvascular systems that are common to the attaching muscles and the bony processes.

The purposes of the present study are to report data regarding —

(1) The blood vascular systems of adult rat and hamster mandibular condylar, coronoid and angular processes, and the muscles which attach to these processes, and

(2) Assessment of the relationship between the vascular systems of muscle, periosteum and medullary bone.

— Materials and Methods —

Six adult male Golden Syrian hamsters and six adult Sprague-Dawley rats were anesthetized with 0.40ML and 0.25ML intraperitoneal injections of Nembutal respectively. The hearts were exposed and 0.25ML of heparin was injected slowly into the left ventricles. Each animal was then perfused with normal saline at 37°C to flush blood from the circulatory system. This perfusant was immediately followed with Microfil Orange at a constant pressure of 150MM of mercury.

Microfil Orange is an injectable self-polymerizing silicone rubber material which leaves a three-dimensional cast of the microvasculature when injected into the vascular system. Perfusion was considered complete when the animals turned a bright orange color. The necks were then ligated tightly to maintain fusate pressure in heads and jaws.

The specimens were stored at room temperature in 10% buffered formalin for two hours to allow polymerization of the Microfil Orange.

Mandibles of four hamsters and four rats were stored overnight in room temperature 10% buffered formalin to complete fixation.

Right and left mandibles of four hamsters and four rats were dehydrated with alcohol, then cleared in Styrene monomer 24 hours for dissecting microscope observations.

The remaining heads were dissected under a dissecting microscope to observe the vasculature of the muscles of mastication and attaching periosteum.

Dried rat and hamster mandibles were also secured for dissecting microscope observations of the surface topography of the condylar, coronoid and angular processes.

Fig. 1
The vasculature of a cleared rat condyle as viewed from the lateral surface, showing an artery that originates at the inferior alveolar artery as it courses toward the osteogenic plexus (O.P.).

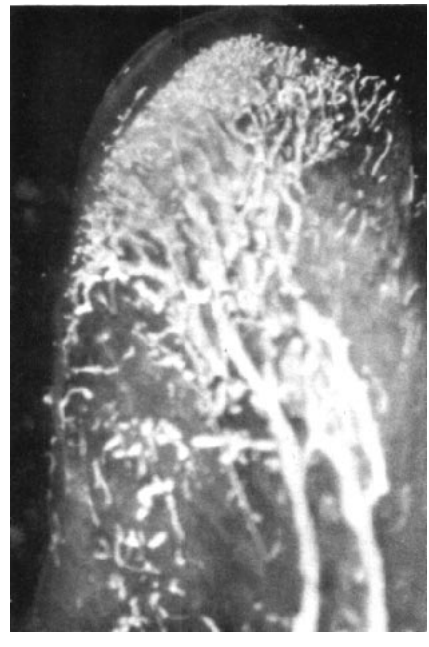


Fig. 2
The vasculature of a cleared rat condyle as viewed from the lateral surface. Note that two arteries originate from the inferior alveolar artery as they course toward the osteogenic plexus.

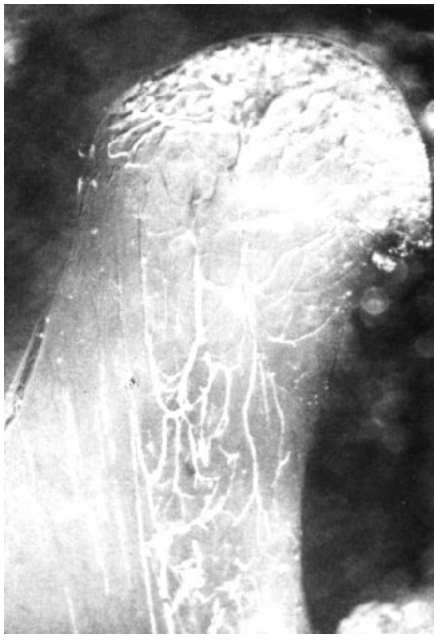
— Results —

Figure 1 shows that the blood vessels supplying the rat condyles are partially derived from an artery originating from the inferior alveolar artery as it courses within the mandibular canal toward the mental foramen. Often, two arteries branch from the inferior alveolar artery (Fig. 2) and course to the osteogenic plexus of the condyle.

The blood vessels supplying the hamster condyle (Fig. 3) differ from the vascular patterns observed in the rat. The arteries that originate from the inferior alveolar artery are small and numerous. As they course toward the condylar cartilage, they anastomose with other vessels that appear to be coursing away from the diffuse vascular plexus of the osteogenic parts of the cartilaginous layer.

These observations suggested that the vascular plexus of the osteogenic tissues in the hamster could not have been totally supplied by the vessels that originated from the inferior alveolar artery, and further, that blood vessels from an external source anastomosed with the vascular

Fig. 3
The vasculature of a cleared hamster mandibular condyle as viewed from the lateral surface. No definitive arteries originate at the inferior alveolar artery, but note the anastomoses with arteries from the osteogenic plexus.



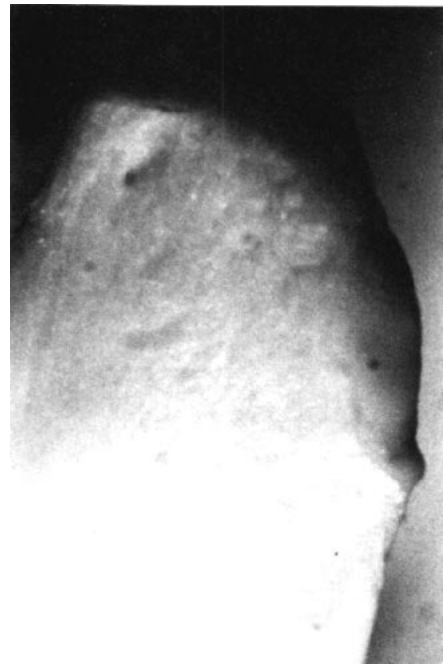


Fig. 4
The lateral surface of the condylar process of a dried rat mandible, showing numerous cortical foramina.

plexus of the condylar cartilage osteogenic layer.

The dissections of two animal condyle heads showed numerous blood vessels originating from the arteries that supply the inferior head of the lateral pterygoid muscles. Arterioles branched to supply the overlying periosteum, and small periosteal vessels coursed through cortical formamina to anastomose with the underlying medullary vasculature. These observations were supported by dissecting microscope studies of the cortical surfaces of rat and hamster condyles (Fig. 4).

Medullary blood vessels of the coronoid processes showed no apparent anastomoses with vessels originating from the inferior alveolar artery (Fig. 5). The medullary blood vessels were continuous with those of the periosteum which, in turn, originated from the arteries supplying the temporal muscles.

Gross and microscopic dissection of the temporal muscle blood vessels and the surface topography of the coronoid processes verified the continuity of the muscular, periosteal, and medullary vascular systems.

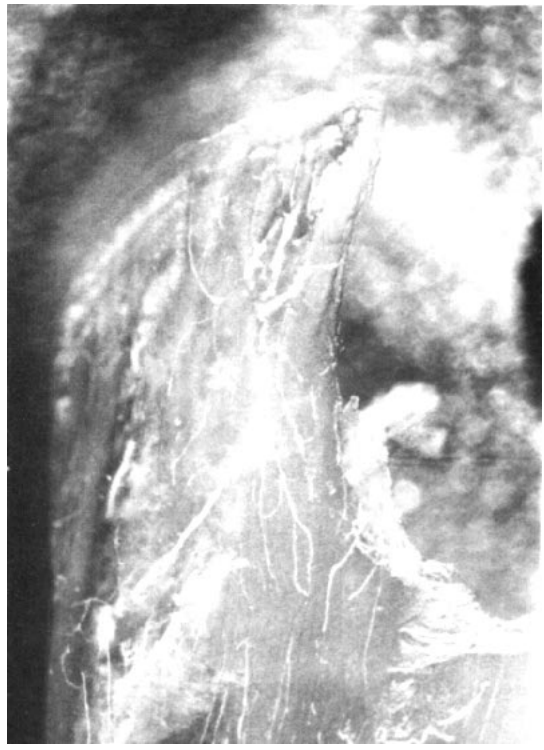


Fig. 5 The vasculature of a cleared hamster mandibular coronoid process as viewed from the lateral surface. Note the paucity of perfused blood vessels.

The medial and lateral surfaces of the rat and hamster angular processes have numerous cortical foramina (Fig. 6) which in life are occupied by blood vessels that originate in the overlying periosteum and muscles. Dissections of the medial pterygoid and masseter muscles supported these conclusions.

The medullary blood vascular systems of the angular processes (Fig. 7) are similar to those of the coronoid processes. These medullary blood vessels have few anastomoses with vessels that originate with arteries from the inferior alveolar artery.

— Discussion —

The results of this research show the vascular supply of the hamster and rat condyle to be derived from an artery (or arteries) originating from the inferior alveolar artery (Dempster and Enlow 1959) and partially from vessels that originate from the vasculature supplying the lateral pterygoid muscle (Castelli 1963).

The muscle vessels branch to supply the periosteum, where they branch to course through numerous cortical foramina and finally anastomose with the underlying medullary vessels.

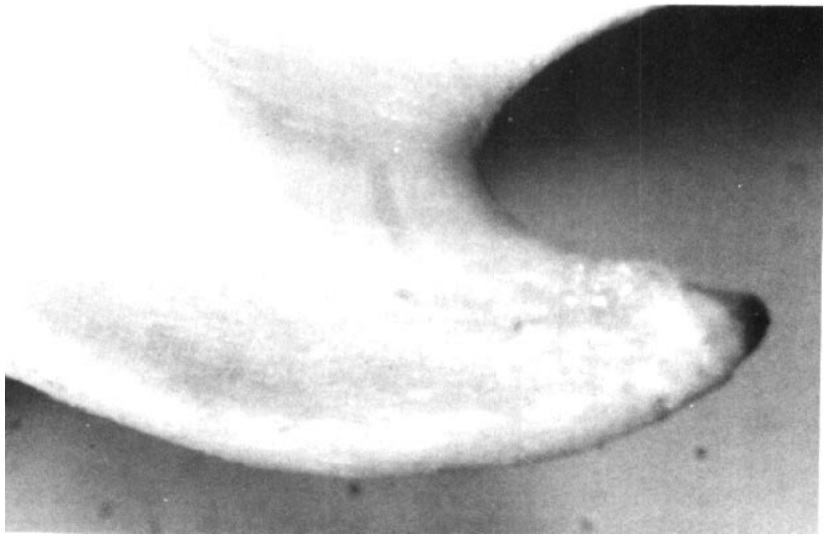


Fig. 6 The lateral surface of a dried, left rat mandibular angular process showing numerous cortical foramina.

Fig. 7 The vasculature of a cleared angular process of a hamster mandible as viewed from the lateral surface.

The coronoid and angular processes have similar vascular systems, except that no anastomoses were observed with vessels from the inferior alveolar artery.

The present study also confirms the data reported in previous investigations (Moss and Moss-Salentijn 1978, Castelli 1963, Zucman 1960, Myrhage 1977, Whiteside and Lester 1978). Collectively, these show the blood vessels of the periosteum to be continuous with those of the muscle and medullary vasculatures. Thus, alterations within the intramuscular and periosteal vasculatures may cause changes in the medullary vasculatures (Hudlicka and Renkin 1968).

The work of Moss-Salentijn (1978) showed that sectioning the femoral and/or sciatic nerve(s) causes a short-term acceleration of growth in the proximal tibial growth plate of the rabbit. The observed short-term increase in vascular density within the medullary bone of the diaphysis following denervation may have been the controlling factor in the accelerated growth of the tibial metaphyseal cartilagenous osteogenic layers.

Similarly, during the embryonic development of the condyle, the vascular complex of the osteogenic layer of the condylar cartilage must increase in complexity as the embryonic condylar cartilage grows interstitially and develops hypertrophic calcified spicules which then provide the scaffolding for initial deposits of osteoid (Furstman 1963, Youdelis 1966). It is assumed that the pluripotential perivascular mesenchyme supplies the cells that differentiate into bone-forming membranes. If this assumption is proven, the importance of vascular systems will reach unparalleled importance in bone morphogenesis and remodeling; however, systematic experimental data are still required.

The existing experimental data (Washburn 1946a and b, Avis 1959, Burke and McNamara 1979, Moss-Salentijn 1978) verify the required interactions between muscle and function for the origin and growth of the mandibular bone processes.

Of equal importance, Washburn (1947) was able to identify three classes of morphological bony features;

(1) those which never appear unless muscle function is present,

(2) those which may be self-differentiating but require the presence of muscle function to remain, and

(3) those which are largely independent of the muscles but happen to be associated with them.

Thus, it is likely that genetic controlling factors are also operating. Genetic factors, in combination with biomechanical and hormonal growth factors, affect the differential growth of the bone membranes that remodel bone tissue and provide the metabolic byproducts that remodel bones to the size and shape expressing the phylogenic history of the species. This process requires the recognition of specific remodeling fields.

A complete understanding of the remodeling problem requires an assessment of the amount of bone tissue that is the product of the proliferation of osteoprogenitor cells and the amount of bone that can be attributed to the migration, induction and differentiation of primordial mesenchymal cells.

Osteogenic proliferation results from the response of the periosteum and endosteum to bone-derived growth factors (BDGF) (Urist 1981). The BDGF initiate the overt stages of remodeling by acting on morphologically differentiated bone cells.

By contrast, there are bone morphogenic proteins (BMP) (Takagi and Urist 1982, Glowacki et al. 1981) that initiate disaggregation, migration, reaggregation and proliferation.

The end result is the covert cytogenic differentiation phase resulting from the

interaction of intra- and extra-skeletal influences. Bone development begins with a morphogenic phase (the covert phase) and ends with the cytogenic phase (the overt phase). Thus RMP and BDGF are coefficient, and the resulting pattern of the bone tissue is established by the positional relationships of cells in three dimensions.

Other hydroxyapatite products appear to induce bone formation in a similar manner. Since blood vessels are the primary source of undifferentiated mesenchymal cells, bone remodeling appears dependent on an adequate blood supply, particularly during the covert stages of bone remodeling.

The genomic expression of bone membranes during normal and abnormal functional stresses or other unknown stimuli is of importance to the physical anthropologist who seeks to understand variations in bone morphology, to the orthodontist who seeks to improve or correct the dental and skeletal contributors to occlusion, and to the surgeon who seeks to normalize abnormal or atypical facial form.

The observation that a part of medullary blood supply to the mandibular processes originates from the muscle and periosteal blood vessels has clinical significance. Muscle-stripping surgical procedures temporarily compromise the medullary blood supply to the affected process, with as yet unknown effects.

Theoretically, stripped bone tissue with a compromised vascular system becomes analogous to a bone graft with dead or dying osteocytes and endosteal membranes. When the vascular system is restored, the bone tissue becomes a scaffold into which new blood vessels proliferate and on which new bone is deposited by the newly-differentiated bone membranes. The process resizes and reshapes the bone tissue to conform with the new functional stresses, as is frequently observed clinically.

It is, therefore, of great importance that clinicians be provided with a precise data base that describes the role of the vascular system in muscle stripping, grafting, and bone remodeling in general. The present experiments represent a beginning. However, the large particle sizes of Microfil may have prevented perfusion of all patent blood vessels. Recognizing that it is impossible to perfuse the entire microvascular bed, the design of appropriate experiments using a material with smaller particle size such as Mercox Resin may enable more detailed studies.

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