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MICROSTRUCTURAL EXAMINATION: POSSIBILITIES FOR SKELETAL ANALYSIS

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Introduction

As pointed out in the introductory paper, myths and fallacies concerning what skeletal analysis can and cannot accomplish abound among both biological anthropologists and archaeologists. Often, biological anthropologists are not aware, or do not use, existing methodologies which are applicable to the analysis of archaeological bone. Archaeologists, working within a broader analytic framework, may not be aware of the total value of the boney specimens they unearth. It therefore the task of biological anthropologists 1) to be aware of the multitude of techniques which are available for skeletal analysis, and 2) to relay this information to archaeologists. The most information possible can then be gleaned in the recovery and final analysis of human remains.

While a number of techniques exist for the analysis of pre-historic bone on a macroscopic level, methods of microscopic study have been minimally utilized. Microscopic analysis of archaeological bone is a relatively new approach involving thin-sections of bones and preparation of slides. A premature and negative assessment of this type of analysis for anthropological data has kept it from becoming a widely used research method for biological anthropologists. A recently written book on skeletal analysis had only a very small section on microscopic analysis (Ubelaker, 1978). Exclusively covered was the use of microanalysis as an aging technique, yet it was ignored as a method applicable to broader anthropological concerns. Putschar (1966:58-59) stated that "One should not . . . expect too much help from the microscopic examination . . . since diagnostic microscopic bone patterns are rare" (pp. 58-59). The quote from Blumberg and Kerley (1966: 54) that "No one pattern is diagnostic" reflects the general attitude towards microscopic analysis of bone for broader applications.

In response to this attitude, the study of archaeological bone as a physiological tool for understanding process is being undertaken by several researchers, for example, Huss-Ashmore (1978), Martin and Armelagos (1979), Ortner (1979), and Stout (1979). Findings from these studies modify earlier statements.

While specific patterns of disease may not be immediately apparent, differences in cortical bone remodeling rates do reveal generalized pathological conditions. Diagnostic patterns which are reflected in histologic changes in archaeological bone include conditions such as metabolic disturbances, systemic diseases, osteoporoses, specific nutritional deficiencies, and more generalized dietary stresses. Microscopic analysis can also support and further define diagnoses of macroscopically determined pathologies such as infectious lesions and porotic hyperostosis.

Thus, histological analysis of bone can provide clues to the overall physiological state of an individual prior to the point of death. Since calcified tissue has only a limited number of ways of reacting to stress, defining patterns of changes can provide a dimension of biological information which may not be available from other sources.

Techniques of Analysis

Because bone is in a constant state of remodeling by resorption and deposition, introduction of any stress which seriously affects metabolism may alter these rates. Although actual cells which are involved with remodeling, that is, osteoclasts and osteoblasts, are not preserved, the microstructural result of their activity is (Stout and Teitelbaum, 1976). Clinical pathologists have concentrated much effort on recognizing the essential features that distinguish normal from abnormal bone remodeling rates, and in the process have discovered ways of quantifying the ratio between the laying down of new bone and the removing of the old (Frost, 1966). Morphometric analysis deals with an assessment of the ratios of resorption to deposition, turnover rates, and measurement of the dimension and size of various microstructural components of bone.

Many features of bone microstructure are often preserved and readily detected in archaeological remains (Stout, 1978). Standard procedures for making thin-sections include the removal of a cross-section of bone from the archaeological specimen. This section can then be embedded for structural support, mounted, and ground to the proper thickness (that is, 100 microns) for microscopic analysis (Stout and Teitelbaum, 1976). While the four

represents a readily available and sturdy bone to examine, others have successfully used the rib and tibia. Haversian systems or osteons, lacunae, resorption spaces, interstitial lamellar bone, cement lines, Howship's lacunae, and other features of microstructure are easily discernible if post-mortem demineralization has not occurred (Stout, 1978).

The sections of bone can be examined as is, or stained, or microradiographed to enhance the microscopic structures under investigation. Staining of bone sections can highlight osteons which are in the process of forming (Villanueva, 1974). Microradiographs have the additional advantage of readily differentiating mineralized (or older bone) from non-mineralized (or newer bone). An area of high mineral content, such as interstitial lamellar bone, appears white or off-white. An area of low mineral content appears off-black, but one devoid of any mineral component appears as a pure black region. Rates of osteon remodeling can be examined by obtaining the frequencies of resorption spaces, which are characterized by rough edges, and forming osteons, which are characterized by low density and a relatively large Haversian canal (Ortner, 1975).

Newly forming foci of bone are clearly evident. Forming osteons can be defined on the basis of size and degree of mineralization. Resorption spaces are also clearly observable. Quantification of the ratio between resorption foci and formation foci offers an excellent reference point for assessing normal development and pathological processes.

Application

Microscopic examination can be successfully employed to address anthropological problems on several levels. Pioneers in microstructural analysis such as Kerley (1965) and Bouvier and Ubelaker (1977) have profitably used cross-sections of long bones to determine age at death of individuals from archaeological populations. Microscopic determination of age contributes to the use of paleohistologic techniques, but the approach itself is limited in scope and applicability. Conditions which alter the normal remodeling rates of bone, for example juvenile osteoporosis and nutritional deficiencies, can substantially increase the chance of error in age estimation. Stout (1978) reports several instances in microscopic studies where metabolic disturbances went undetected resulting in gross error in aging.

Another example of the application of microanalysis to an anthropological concern is that of paleopathological examination and diagnosis. There are a number of pathological skeletal conditions

which are amenable to microscopic analysis. Specific diseases that can be diagnosed from archaeological bone include hyperparathyroidism, diabetes, arthritis, tertiary syphilis, osteogenesis imperfecta, Paget's disease, and vitamin D-resistant rickets (Jowsey, 1963; Frost, 1966). While these examples are rare in prehistoric samples, more generalized pathological conditions, such as nutritional disturbances and osteoporosis, are also important biological phenomena to examine.

Several researchers believe that malnutrition is detectable in the skeletal system (Frost, 1966; Huss-Ashmore, 1979; Ortner, 1976). Age controlled samples from temporally sequential archaeological populations have been looked at. A preliminary investigation by Stout (1979) of two prehistoric skeletal populations from the Middle and Late Woodland periods has shown that the later group had a much higher rate of bone remodeling. Richman et al. (1979), in a study of three prehistoric populations from differing environments, has also shown differing rates of remodeling. Both of these preliminary studies point to a possible dietary explanation for the differing remodeling rates. Deficiencies in protein and calcium do produce increased remodeling rates (Jowsey, 1963). And, as is frequently pointed out, maize and other common agricultural staples are poor sources of both protein and calcium. However, in this new area of study, we must be careful not to make hasty conclusions based on increased remodeling rates only. Rather, there must be a careful coordination of all available information, such as remodeling rates, trace element analysis and dietary reconstruction, in order to make sound interpretations concerning nutritional stress. As pointed out in the previous paper, nutritional stress must be viewed as an interface between environmental and biological variables (Huss-Ashmore, 1979).

Another way in which histological analysis can aid in archeological interpretations is by doing a populational analysis of a generalized pathological condition, such as osteoporosis. Osteoporosis, defined as a decrease in bone mass, has been studied in both modern and prehistoric populations, but the etiology remains unknown. Severity of osteoporosis in long bones can be measured by porosity and thickness of the cortex. While several researchers have studied the manifestation in prehistoric samples at the gross level (Armelagos et al., 1972; Ericksen, 1976; Perzigian, 1973), no explanation of the underlying histological processes has been attempted. While osteoporosis is a normal process in the aging of adults, Garn (1970) also notes the occurrence of juvenile osteoporosis. Rapid resorption at the endosteal surface with slower formation and mineralization at the subperiosteal surface is typical of the osteoporotic process.

The sample used for this study consisted of 74 adults aged from 20 to 50-plus years. There were 40 females and 35 males. These adults were recovered from an X-Group cemetery in Sudanese Nubia. The X-Group horizon is dated at A.D. 350-550 and presents a phase in Nubian cultural development following the decline of the Meroitic kingdom, and before the rise of Christianity. The X-Group was made up primarily of agriculturalists utilizing the flood plains of the Nile River. Archeological evidence suggests that the X-Group population was an independent group relying on local resources.

Patterns of cortical growth and maintenance were determined from a microradiographic analysis of femoral sections. The bones were embedded, sectioned, and microradiographed according to procedures described by Armelagos et al. (1972:117). Both macroscopic and microscopic examinations were employed in a morphometric assessment of cortical dynamics.

Six measurements of the cortical thickness were made on the contact microradiographs. Cross-sectional area of the cortex was then computed according to the method as reported by Carlson et al. (1977). Eight equidistant microscopic fields were assigned on each specimen. The average number of osteons in each viewing field was 30, thus at least 200 osteons were assessed for each individual, thereby reducing the chance of error induced by random osteon variability (Stout, 1978). The fields were selected so that they alternately covered the outer periosteal and the inner endosteal envelopes.

To understand the underlying cortical processes involved in the increase of osteoporosis with age, bone formation and resorption ratios were determined. In addition to determining rates of deposition and resorption, assessment of mineralization and density was undertaken. Degrees of mineralization were measured by contrasts in density. This provided the means for assessing the various phases of the remodeling process.

Males and females exhibit a marked difference in states of bone loss as measured by cortical thickness (Figure 1). Cortical thickness for Nubian males follows a trend very similar to that found in studies of modern (Garn, 1970) and prehistoric (Erickson 1976) populations. Females show an initial increase, with a rapid decrease following the 40th year. This initial increase from the third to the fourth decade is not paralleled by modern populations (Garn, 1970), where the trend is a less severe decline from age 20 until death.

The relationship of cortical area to age was determined (Figure 2). Cortical area is a direct function of both cortical thickness and periosteal diameter. In addition, cortical area provides a means for assessing intercortical porosity. Similar trends for area are found. Females again show an increase to 30, and then a progressive decline. The male cortex is more consistently maintained throughout life, while female loss is more steady and severe.

Microscopic analysis clarifies the processes which resulted in bone losses. The average number of forming osteons and resorption spaces was calculated. The number of forming osteons and resorption spaces is significantly different ($p < .05$) between males and females in the third decade. Females exhibit more resorption and formation. After age 30, there is no significant difference in the frequencies. These trends support the findings at the macroscopic level. Females in the 20-29 age class are showing increased remodeling rates relative to the rest of the population.

A further analysis of the histological differences between the inner and outer portions of the bone showed interesting trends. On the outer or periosteal portion, there is approximately a 2:1 ratio between formation and resorption (Figure 3). Trends and resorption are similar for males and females. The trends in formation are much more variable, suggesting a reaction to some type of physiological stress in the 20-29 age class for females.

It is at the endosteal surface that the most dramatic differences in resorption and formation are found. While periosteal resorption remained virtually unchanged, endosteal resorption increased substantially for third decade females.

A discussion of these findings follows. Females exhibit an earlier and more severe cortical loss with age. While many other studies on archeological populations demonstrated similar results (Dewey et al., 1969; Ericksen, 1976; Perzigian, 1973), an analysis which combines microstructural examination with macroscopic observations is essential for understanding the underlying processes.

Consistently, the results showed a premature bone loss for females in the 20-29 age class. Females surviving this age class exhibit increased amounts of bone with more even rates of mineralization and resorption. This pattern suggests a stress being placed on females in their early adulthood. Traditionally, this is the period of childbearing and childrearing. Multiparous females are known to be placed under added nutritional stress in

terms of fetal and newborn requirements which are often taken from the mother's reserves (Mensforth et al., 1978). Lactation alone uses up to 300 mg of calcium and 100 kilocalories per day (Worthington et al., 1977). Various other studies of prehistoric populations which are agriculturally based have shown them to be under chronic nutritional stress due to the narrow food base (Cassidy, 1974; El-Najjar et al., 1976; Lallo, et al., 1977; Mensforth et al., 1978). The synergistic effects of nutritional stress, pregnancy, lactation and workloads may be producing the increased remodeling rates and the marked bone loss in this sample of Nubian females. Taken together, these results suggest that the stress of childbearing added to existing physiological stresses have an effect on biological processes such that morbidity and mortality rates are substantially altered. An adaptive mechanism may be at work allowing for the recycling of existing bone when maternal stores are at stake. Formation rates for the Nubian males and females showed extreme variability. These findings are supported by clinical studies which show that remodeling is not static, but rather a dynamic process which is affected by various underlying conditions.

The application of morphometric analyses may shed light on the underlying processes involved not only in prehistoric populations but modern ones as well. Archeological populations present a resource with which to investigate the patterns of normal and pathological development relevant to human behavior. Combined with archeological reconstruction of biocultural adaptation, paleohistological techniques present a method by which data can be accumulated leading to a more complete view of the health and disease status of extinct and extant populations. Techniques for microstructural analysis are available, and if applied systematically, can aid in solving processual problems. Spatial and temporal comparisons of modern and prehistoric populations should clarify some of the variables which affect human adaptability.

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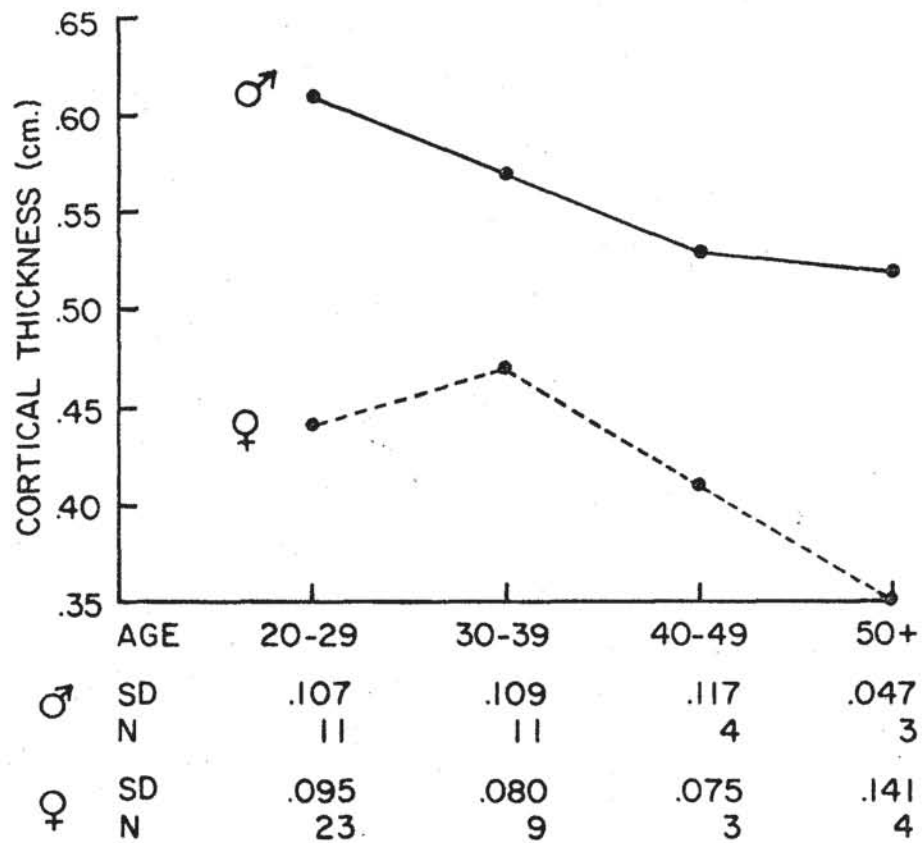


Figure 1. Graph illustrating the difference between male and female cortical thickness.

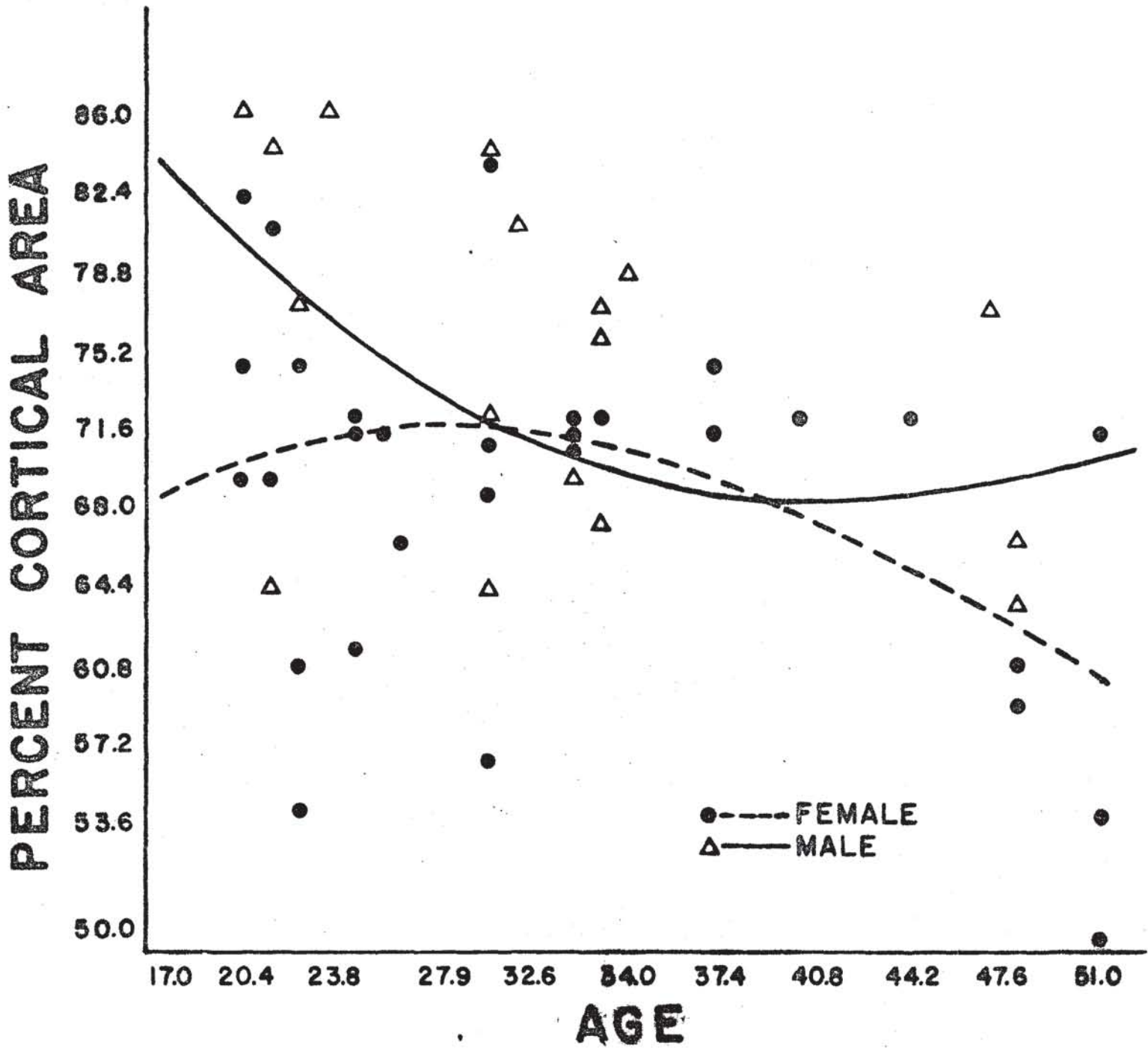


Figure 2. Curvilinear regression showing the trends for male and female percent cortical area.

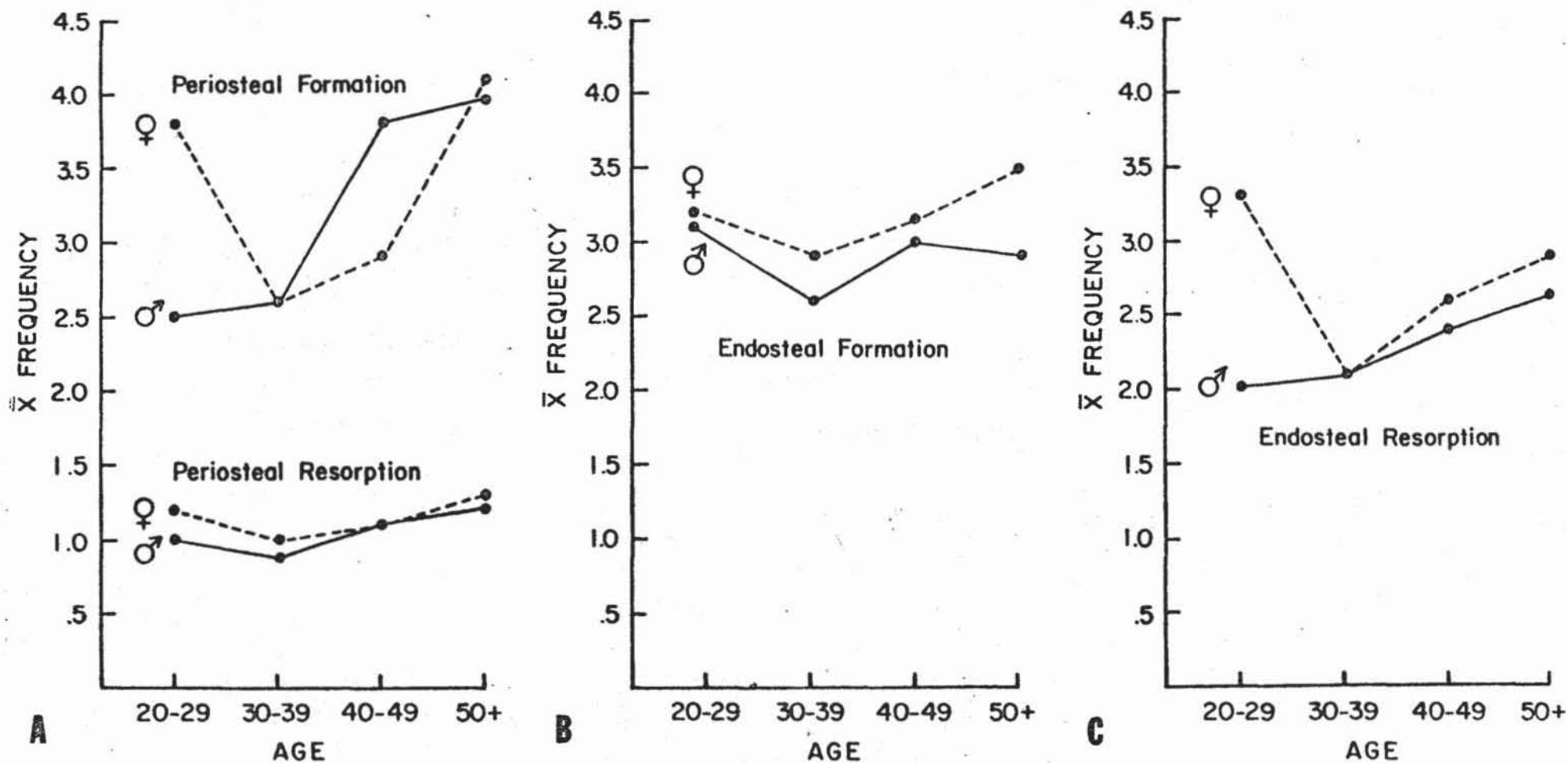


Figure 3. Graphs showing the histological variation in microstructural analysis between the inner and outer (periosteal and endosteal) portions of the sections.