THE SYNTHESIS OF COLLAGEN PHENOTYPES IN ORTHOPAEDICALLY STRESSED MOUSE INTERPARIETAL SUTURE

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CHUNG-SUT YUE

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ABSTRACT

Collagen metabolism is an integral part of the biochemical aspects of fibrous joint remodelling during growth and development and when the joint is physically stressed, as during orthodontic and facial orthopaedic therapy. However, the metabolic behaviour of collagen during such remodelling is largely unknown. The present studies have explored collagen phenotype synthesis during rapid remodelling of fibrous joints.

Mouse interparietal sutures were subjected to tensile stress with implanted helical springs for various periods of time before sacrifice. The ratios of type III collagen in the salt soluble fraction of stressed sutures were found to be significantly higher than those of sham-operated non-stressed controls. Thus, collagen type III ratio could serve as an important parameter indicating the nature of collagen metabolism during rapid remodelling of fibrous joints.

Subsequently a model system which permitted mouse interparietal sutures to be stressed *in vivo* and then labelled *in vitro* as calvaria explant organ cultures was developed, and then verified by comparing its results with those from *in vivo* labelled samples. This system is particularly suitable for the study of factors regulating cellular activity, including collagen metabolism.

The collagen type III ratios in the mouse interparietal suture from newborn to maturity (age study) and when mechanically stressed at five different force levels (force study) were then determined with the methodology developed.

The age study indicated that high collagen type III ratios are closely related to histo-differentiation, morphogenesis and rapid growth of sutural tissues. Also, with reference to the collagen type III ratio, the optimal age for investigations of sutural response to mechanical stimulation in mice was determined to be between nine and ten weeks old.

The force study suggested that tensile stress of low force levels causing minimal tissue damage was best able to initiate the changes in the collagen type III ratio indicating remodelling. The range of the type III ratios associated with the light forces related well with the range which existed during rapid growth and development of the suture and also light forces tended to initiate a more physiological response than heavy forces. When this "force to remodelling" response relationship is better understood, the efficient achievement of orthodontic objectives with no iatrogenic effects may be possible.

DEDICATION

To the Yues

and

All Who Helped Along the Way

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(A) CLINICAL ORTHODONTICS AND FIBROUS JOINTS

The objective of orthodontic treatment is to correct the malocclusion of teeth and the associated deformities of the facial skeleton. Except for the mandible, this is achieved primarily by the controlled mechanical manipulation of the fibrous joints of the craniofacial complex through the process of biologic adaptation termed remodelling (Enlow, 1982). Indeed, remodelling is an inherent biologic activity of fibrous joints that meets the structural and functional requirements of these anatomic entities throughout life.

There is a continuing change of relationship between the tooth and its supporting structures during tooth eruption and between the bones and articulations of the craniofacial skeleton during growth and development. This remodelling continues, though most likely at a much slower rate than under normal circumstances, even after completion of growth in the craniofacial skeleton and after the establishment of dental occlusion. The mechanical stress to the sutural and periodontal fibrous joints during muscle activities of the face as well as during functional and parafunctional activities of the dentition probably require the integrity of this remodelling process of fibrous joints be maintained throughout life. Moreover, according to the Theory of Attritional Occlusion (Begg and Kesling, 1977), teeth continually erupt and migrate mesially in compensation for the loss of tooth material due to occlusal and interproximal wear. It was estimated that the approximate amount of interproximal reduction of tooth material in

each quadrant, prior to the eruption of third molars, equals the mesio-distal width of a bicuspid tooth (Begg and Kesling, 1977). Although these findings were from the study of Australian aborigines who presumably lived as Stone Age man, the progressive increase in lower face height and crowding of anteriors with age in modern man have been attributed to a lack of occlusal and interproximal attrition as a result of softer, modern diets, in combination with a still operational tendency for teeth to erupt occlusally and mesially (Begg and Kesling, 1977). Indeed, the only constancy of the dentition is the continual adaptation of the periodontal supporting tissues (Melcher, 1980).

Thus, fibrous joint remodelling is part of the normal biologic activity throughout life. Orthodontic and orthopaedic manipulation of periodontal and sutural articulations can be essentially visualized as an accelerated version of this normal biologic event. The ultimate clinical aim would be to accelerate this remodelling process at an efficient rate without iatrogenic effects. This would be possible only with a better understanding of the physiology and biochemistry of tissue remodelling at the molecular and cellular levels, and when the sequence of events between the application of mechanical stress and the resultant morphological changes have been better defined. Only then will the understanding of the scope and limitations of orthodontic therapy, as determined scientifically, be possible.

The importance of remodelling can be further illustrated by the pathologic behaviour of fibrous joints, such as premature sutural synostosis (e.g. Apert's, Crouzon and Treacher Collins

Syndromes), delayed sutural fusion and tooth eruption (e.g. cleidocranial dysostosis) and fused periodontal ligament (e.g. tooth ankylosis). The etiology, prevention and treatment of these conditions depend on the understanding of the biology of fibrous joints.

Periodontal ligament and craniofacial sutures are both classified as fibrous joints because of the many characteristics they share, both in terms of structure and biologic behaviour. In order to correlate and to rationalize the extrapolation of findings from the study of one structure to that of the other, a comparison and contrast of the two anatomic entities should be considered.

(B) ANALOGY OF SUTURAL AND PERIODONTAL FIBROUS JOINTS

The histological similarities between the periodontal ligament and the suture were noted by Noyes back in 1934. Since then, many investigators have commented on both the structural and functional similarities of the two structures (Hinrichsen and Storey, 1968; Moffett, 1971, 1973; Storey, 1972; Ten Cate et al., 1977; Nanda, 1978; Melcher, 1980). Both are joints, connecting two adjoining hard tissues by an intervening dense fibrous connective tissue. While sutures act as sites for craniofacial growth, periodontal ligament allows for tooth eruption; and both structures are stress-bearing throughout life, though functional demands to the sutures decrease greatly with the cessation of craniofacial growth. Following trauma or injury, the normal healing processes for both are by repair followed by regeneration (Gilman, 1968) with the complete restoration of the original tissue architecture rather than scar formation (Ten Cate et al., 1976, 1977).

The major differences between the two structures lie in the progressive changes of sutures with age and the important sensory function which is unique in the periodontal ligament.

Following is a detailed comparison of the two structures under the following headings:

(1) Morphology and structure

(2) Physiology and biochemistry

(3) Response to mechanical stress.

(1) Morphology and Structure

Craniofacial sutures have been identified as the intervening fibrous connective tissue between the adjoining membranous bones of the craniofacial skeleton. Moss and Young (1960) introduced the term "sutural area" which includes the fibrous connective tissue together with the contiguous bone to emphasize their continuity and interrelationships. Similarly, it would be more appropriate to consider the periodontal fibrous joints which are comprised of the cementum of tooth, the intervening cellular, vascular and fibrous connective tissue and the alveolar bone as the functioning unit rather than just the periodontal ligament. Thus we have a fibrous tissue connecting two calcified connective tissues in both cases, except cementum and bone are at either end of the periodontal joints, while only bony tissues are found at both ends of the sutural joints. The soft connective tissue portion of both structures consists of various types of cells, fibres, ground substances and blood vessels (Noyes, 1934; Persson, 1973; Melcher, 1980). Although cartilage remnants may be present in sutures of animals and man (Noyes, 1934; Moss, 1954; Pritchard et al., 1956; Griffiths et al., 1967; Bloore et al., 1969; Persson, 1973; Friede, 1975; Hall, 1978b; Al-Bareedi, 1984), they are never found in the periodontal ligament. However, very little is known of the role of cartilage in the suture.

The histological organization of the suture has been described variously by investigators as three layers (Weinmann and Sicher, 1955; Linge, 1972), four layers (Scott, 1954; Droschl,

1975), five layers (Pritchard et al., 1956) or as three layers developing into a single fibrous layer in the adult (Moss, 1954). While Pritchard et al. (1956) found the five-layer picture even in the non-growing adult suture, others (Enlow, 1968; Latham, 1971) viewed the adult suture as a more homogeneous structure composed of coarse collagenous fibres with fewer cells and blood vessels.

These inconsistencies in the description of sutural structure may stem from variations between different species, different ages and maturation stages (Scott, 1954; Kokich, 1976), different sutures, or from different selective staining techniques employed. Indeed, even the fibre pattern within a given suture may vary (Isotupa et al., 1965; Koskinen et al., 1976; Nanda, 1978) and the controversy has not been resolved with the introduction of electron microscopy (Ten Cate et al., 1977).

However, some common ground can be extracted from these various studies. There seems to be a consensus amongst investigators of the existence of a cellular cambial layer, close to each bony margin of a developing suture, which exhibits a high level of bone formation activity (Scott, 1954; Pritchard et al., 1956; Latham, 1971; Linge, 1972; Droschl, 1975; Friede, 1975; Yen and Chiang, in press) and a middle fibrovascular layer, rich in blood vessels and mesenchymal cells and where collagen is much looser in texture and composed of immature appearing fibrils. This middle area has been suggested as the site of cell proliferation and fibre rearrangement (Weinmann and Sicher, 1955; Pritchard et al., 1956; Linge, 1972; Droschl, 1975; Ten Cate et al., 1977), although this point

is still controversial. In addition, Pritchard et al. (1956) ascribed the blood vessels to the middle layer only of their five-layer concept. However, this five-layer pattern was not evident in the morphological observation of the interparietal suture of the mouse (Chiang, 1981). Also, blood vessels were observed in both the middle and paraosseous layers and continuity of these sutural blood vessels to the marrow spaces was observed in rats (Persson, 1973) and mice (Chiang, 1981).

The structure of the periodontal fibrous joints is not without controversy either. It is generally accepted that collagen fibres are embedded in cementum and alveolar bone so that the periodontal ligament provides soft tissue continuity between the two mineralized connective tissues. The controversy arises from the fact that the principal fibres of the periodontal ligament frequently demonstrate a wavy course from cementum to bone. Under the light microscope, it may appear as though fibres arising from cementum and bone are joined in the mid-region of the periodontal space, giving rise to a zone of distinct appearance, the so-called intermediate plexus. Sicher (1942, 1954, 1959) and Weinmann (1955) argued that the concept of the presence of an intermediate plexus was the only one which could adequately explain the adaptability of the periodontal ligament to tooth eruption, mesial drift, occlusal attrition, as well as functional and parafunctional stress. Theoretically, it was achieved by an unsplicing and resplicing of the fibres at the intermediate plexus. Subsequent studies on guinea pig (Hunt, 1959) and spider monkey molars (Goldman, 1962) as well as continuously erupting rat inci-

sors (Hindle, 1967) seemed to indicate there is evidence for the existence of an intermediate plexus. This conclusion has received some support from Melcher (1967) who found that in the continuously erupting incisor, the fibres on the alveolar bone side of the ligament remained stationary, while those on the cemental aspect of the ligament are carried incisally by the tooth, suggesting that the ligament is remodelled in an intermediate zone. However, the existence of such a plexus has been disputed by other investigators. Eccles (1959), Bernick (1960) and Trott (1962) either did not mention or disputed the development and existence of an intermediate plexus in their studies on the development of the periodontal ligament of rat molars. By use of radioautographic techniques, no evidence of higher turnover of [^{3}H]-proline in the intermediate plexus zone of the periodontal ligament than elsewhere could be found in mouse (Stallard, 1963) or rat molars (Crumley, 1964). Melcher and Correia (1971) found in the reactivation of limited eruption of rat molars, some remodelling of fibres of the periodontal ligament probably occurs throughout the width of the periodontal space, but that these processes are most active in the portion of the ligament adjacent to the alveolar bone. Zwarych and Quigley (1965) believed the intermediate plexus to be an artefact arising out of the plane of section. This may be due to the fact that the principal fibres may run from one bundle to another and with smaller diameter fibres forming a meshwork and coursing in all directions and forming anastomosing relationships with the principal fibres (Shackleford, 1971).

Despite these controversies, Ten Cate and co-workers (1976,

1977) questioned the wisdom of further structural studies of fibrous joints. Instead, they suggested an alternative approach, namely questioning how the structural elements of fibrous joints respond to and fulfill their architectural, physiological and functional demands during normal growth and development and when artificially stressed. That is, how do sutures and the periodontal ligament ensure their structural and functional integrity throughout life. Obviously, this does not resolve the controversy of histological details of sutural or periodontal structure. However, it does offer a functional approach to explaining the morphological observations.

Ten Cate and co-workers (1976, 1977) suggested that rather than try to resolve the issue of the number of cell layers in the suture or the arrangement of collagen fibres within it, it would be more meaningful to delineate components according to functional criteria. The suture, and similarly the periodontal ligment, can be best considered as consisting of two cell populations: the osteocytic and the fibrocytic series which have the ability to remodel the tissues they form (Ten Cate, 1972; Deporter and Ten Cate, 1973; Listgarten, 1973; Ten Cate and Deporter, 1974, 1975; Ten Cate and Syrbu, 1974; Garant, 1976). The sutures and the periodontal ligament are identical in this respect. In addition, one can postulate the existence of progenital cells (Melcher, 1976, 1980; Hall, 1978a) which undergo mitotic divisions to replace aging differentiated cells (Leblond et al., 1959; Ten Cate et al., 1976; Everts and Beertsen, 1978) or in response to a sudden increase in demand for functional cells, e.g. as a result of

trauma or stress (Melcher, 1976; Gould et al., 1977; Ten Cate et al., 1977; Chiang, 1981). The presence of proliferating cells in the rat periodontal ligament is well established (Messier and Leblond, 1960; Jensen and Toto, 1968; Weiss et al., 1968; Roberts and Jee, 1974). These mitotically active cells presumably represent a population of progenitors capable of generating functional fibroblasts, osteoblasts and cementoblasts in response to local demands (Melcher, 1976, 1980; Hall, 1978a). However, little is known about the progenitor cells. It is not known whether a single population of progenitor cells gives rise to all of the specialized synthetic cells or if there are a number of populations, each of which gives rise to a different specialized cell (Melcher, 1976, 1980; Hall, 1978a).

Ten Cate and co-workers studied the periodontal ligament undergoing physiological tooth movement (Ten Cate et al., 1976), and rat interparietal sutures during rapid growth and development Ten Cate et al., 1977) and when subjected to tensile stress (Ten Cate et al., 1977). They found that the two structures respond similarly to local functional demands by continuing synthesis and degradation of the extra-cellular components until the structural arrangement satisfies the functional environment experienced. Thus the dynamics of sutural and periodontal morphology and their response to functional or externally imposed demands are probably reflections of the metabolic activities of the cellular components.

The main extra-cellular component of both fibrous joints is collagen and can be identified as collagen fibres in histological

sections.

The principal fibre arrangement of the periodontal ligament follows a regular pattern depending on its location along the root surface of the tooth (Ciancio et al., 1967; Melcher, 1980), while that of the sutures are so variable even within the same suture, no definite pattern can be described. Koskinen and co-workers (1976) suggested that the fibre patterns of sutures were reflections of local demands. The non-linear configuration of sutures and the existence of bevelling and bony projections suggest that different sutures and indeed, different parts of the same suture may experience mechanical stress of different direction and magnitude (Nanda, 1978). The direction of the fibres has been interpreted to indicate compression, tension or shear between two adjoining bones (Prahl, 1968; Herring, 1972). The periodontal ligament throughout life and the sutures of young animals can be seen as a dynamic biologic tissue capable of undergoing continuous remodelling. Thus, it follows that the structure of any suture is in fact essentially similar and that the detailed descriptions of differing fibre orientations and vascular distributions reflect no more than differences in the functional state of the structure at any given time rather than immutable characteristics of an anatomically defined structure (Ten Cate et al., 1977; Nanda, 1978). Thus, the apparent differences in structural arrangement of collagen fibres in sutures and the periodontal ligament should be considered no more than the result of structural manifestation of differences in local functional requirement. As such, they probably will respond similarly when subjected to a similar change in

functional environment. Indeed, this concept will be expanded in a later section.

The fibrocytic series of cells is also responsible for the synthesis and maintenance of the other major extra-cellular component of connective tissues, namely, the ground substance which consists mainly of proteoglycans (acid mucopolysaccharides) and glycoproteins. Both groups are composed of proteins and polysaccharides, but of different types and arrangements. (For detailed structures, see Silbert, 1978). The integrity of the ground substance is essential if the cells of the connective tissue are to function properly as all anabolites reaching the cells from the micro-circulation and all the catabolites passing in the opposite direction must pass through the ground substance.

Mathews (1965), Toole and Lowther (1968) and Plecash (1972) showed that sulphated glycosaminoglycans were involved in the formation and orientation of collagen fibres. Glycosaminoglycans were also reported in association with developing transalveolar fibres in mouse (Johnson, 1981). Mucopolysaccharides are involved in osteogenic activity of developing sutures (Persson, 1973) and in physiological root resorption of human deciduous teeth (Alexander and Swerdloff, 1979). A proteoglycan carrier system has been described by Hay (1978) and Olsen and Low (1980) and may function in the transport of tropocollagen to the remodelling fibres. In addition, the possible role of ground substance in joint lubrication, hydration and even in the control of cyto-differentiation of fibroblasts or chondrocytes from mesenchymal cells has been suggested (Silbert, 1978).

Turnover activity of ground substance has been demonstrated by Baumhammers and Stallard (1968) in radioautographic studies of mouse molars using radioactive sulphur. However, no information is available concerning ground substance metabolism during tooth eruption, or sutural and periodontal ligament remodelling.

The major difference between the sutural and periodontal joints seems to be the progressive structural changes with age of the sutures (Moss, 1954; Scott, 1954; Pritchard et al., 1956; Persson, 1973; Kokich, 1976), while the periodontal ligament, once developed, essentially remains unchanged throughout life.

In general, the suture shows age changes in cellular element, vascularization and collagen fibres. The cellular element in the suture decreases with advancing age (Kokich, 1976) and also becomes less active (Ten Cate et al., 1977). But, at least in the monkey, the cells appeared to retain the ability to respond to extrinsic force applied to the suture, though less responsive initially (Brandt et al., 1979).

The concentration of collagen fibres also tends to decrease with advancing age with the fibre bundles becoming irregularly spaced (Miroue and Rosenberg, 1975; Kokich, 1976). However, with age, the orientation of the fibre bundles becomes more regular (Kokich, 1976; Koskinen et al., 1976).

Both Pritchard and co-workers (1956) and Persson (1973) have described the increasing presence of thin walled sinusoidal vessels with age in cranial sutures but with no explanation of its significance. Persson (1973) also noticed a regression of capillary density, though the vascular pattern varied only slightly

with age.

Interdigitations of sutural bone margins increase with age due to the increase in length and number of bony projections into the sutural area (Melsen, 1975; Miroue and Rosenberg, 1975; Kokich, 1976), presumably to increase the strength of the fibrous joint.

However, the average age for suture synostosis is still highly controversial as it varied widely among investigators (Sicher, 1965; Bjork, 1966; Scott, 1967; Latham, 1971; Miroue and Rosenberg, 1975; Kokich, 1976). This may be due to differences in methodology and animal models used by each investigator and differences in animal species, sutural sites or functional state.

Sutural synostosis, unless grossly premature as in Apert's and Crouzon and Treacher Collins syndromes, is considered to be a normal physiological process. Tooth ankylosis, on the other hand, is always considered to be pathologic.

Little is known of the qualitative and quantitative changes occurring with age within the periodontal ligament except that it may become narrower. This change is probably related to the force applied to teeth. The heavier the load for a tooth, the wider is its ligament. Older people presumably have a less powerful bite with the result that the teeth are less stressed and their periodontal ligament becomes narrower (Osborn and Ten Cate, 1983a).

Also the thickness of the periodontal ligament varies in different individuals, different teeth in the same person and different locations on the same tooth (Coolidge, 1937) and seems to be related to the amount of occlusal stress. In general, the perio-

dontal ligament is the thinnest in the middle region of the root as this is the center of physiologic tooth loading, and the widest at the alveolar crest and at the apex where displacement due to physiologic tooth loading will be the greatest.

The interrelationship between structure and function can be further illustrated in erupting teeth, where the fibre bundles for the periodontal ligament thicken appreciably with the development of functional occlusion (Bernick, 1960; Grant and Bernick, 1972). Johnson (1981) made similar findings in his study of transalveolar fibre development in the mouse.

On the other hand, with loss of function, much of the extracellular substance of the ligament is lost and the width of the periodontal space is also decreased, possibly because of the decrease in biologic activities in the cellular component. The process is reversible if function is returned to the tooth, but the precise nature of the stimuli that control the change in activity of the cells is unknown (Cohn, 1966).

Thus, it may seem that the progressive change with advancing age differs greatly between sutural and periodontal joints, especially viewed from the structural aspect. However, functional demands on the sutures decrease greatly with the cessation of craniofacial growth. As the animal ages, the primary function of sutures is that of joining adjacent bones, and that of a stress-bearing site for functional forces becomes less and less important. However, at least in young adult animals, sutures still retain the potential to respond to extrinsic applied forces, though the tissue is less responsive initially (Brandt et al.,

1979). Yet, as long as teeth are in functional occlusion, the periodontal ligament is always stress-bearing. This may explain why the rates of collagen synthesis and degradation appear to decrease greatly with age in other connective tissues, but fails to do so in the periodontium where collagen is rapidly degraded and replaced throughout life (Narayanan and Page, 1983a).

Even so, with decrease in occlusal force due to old age or when a tooth is in a hypofunction state, morphological changes of the periodontal ligament are evident (Cohn, 1966; Osborn and Ten Cate, 1983a). Therefore, the difference in changing functional demands with age could be the main reason for the difference in structural changes with age for the sutural and periodontal joints.

Another major structural difference between the sutural and the periodontal fibrous joints is the presence of cementum in the periodontium. Cementum is a specialized connective tissue that shares some physical, chemical and structural characteristics with compact bone. Unlike bone, however, human cementum is avascular and can be either cellular or acellular. Parallel with the osteocytic and fibrocytic series of cells, there is the cementocytic series of cells: cementoblasts, cementocytes and cementoclasts, plus progenitor cells. In contrast to the alternating resorption, formation activity of bone, cementum is not resorbed under normal conditions. Instead, new layers of cementum are continuously being deposited to keep the attachment apparatus intact. Deposition of cementum by cementoblasts appears to be a slow continuous process and resorption is not a regular occurrence

(Armitage, 1980). However, cementum resorption can occur after trauma or excessive occlusal forces. Usually, these can be repaired by deposition of new cementum by cementoblasts (Armitage, 1980; Harry and Sims, 1982).

Cementum is more resistant to resorption than is bone, and it is for this reason that orthodontic tooth movement is made possible. However, the reasons for this difference are still unknown and the following suggestions can only be speculative:

(a) Only deposition, but not resorption of cementum, is a normal physiological process, while continual deposition and resorption is characteristic of osseous tissues.

(b) The difference in the resistance of bone and cementum to pressure may be caused by the fact that bone is richly vascularized, whereas cementum is avascular. The degenerative processes are much more easily affected by interference with circulation in bone, whereas cementum with its slower metabolism (as in other avascular tissues) is not damaged by a pressure equal to that exerted on bone.

(c) Cementum avascularity may limit the presence of osteoclastic cells which may migrate via the vascular system.

The goal of orthodontic management should be to exploit this difference between cementum and bone to the fullest for efficient tooth movement with the minimal undesirable sequelae.

(2) Physiology and Biochemistry

Sutural and periodontal fibrous joints serve a number of similar functions. They provide continuity between adjoining hard connective tissues. While sutures act as areas of growth for the craniofacial complex, the periodontal ligament allows for tooth eruption. Both possess the unique biologic process of remodelling and are capable of regenerating from injury or trauma instead of forming scar tissue. These properties are essential to maintain their capacity for adaptation to growth and stress. However, only the periodontal ligament also serves the very important proprioceptive sensory function.

The sutures as areas of growth have been demonstrated by biometry (Gans and Sarnat, 1951; Selman and Sarnat, 1955; Bjork, 1966); by histology (Moss, 1954; Pritchard et al., 1956); by vital staining (Moore, 1949; Massler and Schour, 1951; Isotupa et al., 1965; Hong et al., 1968; Hoyte, 1971; Cleall et al., 1971; Cleall, 1974), and by radioautography (Leblond et al., 1950; Dixon, 1961; Young, 1962; De Angelis, 1968; Persson, 1973; Persson and Roy, 1979; Yen and Chiang, in press). However, whether they are growth sites reactive to local structural and functional demands or whether they are growth centres with intrinsic, active and autonomous growth force which can separate adjacent bones, has long been a subject for debate.

For the growth centre theory, Moore (1949), Weinmann and Sicher (1955) and Sicher (1965) suggested the expansile force of cellular proliferation within the sutural tissue, analogous to the

epiphyseal growth plate of long bones, to be the basis for bone separation. Maturation of the collagen macromolecules in the fibres may also provide separating forces (Prahl, 1968).

Moss (1962) suggested that the craniofacial sutures are in an environment of various forces including functional forces (e.g. mastication, deglutition and respiration) and force from growth (most notably the expansile force caused by the growth of the brain). Thus, growth at the sutures were considered to be reactive and merely growth sites rather than growth centres.

The dependence of sutural growth on local environmental forces has been demonstrated by isolating craniofacial sutures in tissue culture or by transplantion to presumably non-growing environments in vivo with termination of sutural growth (Moss, 1957; Watanabe et al., 1957; Prahl, 1968; Lewin and Irwing, 1970). In addition, when a suture is transplanted to a site where growth is rapid and forces exist, its growth may be greater than normal (coronal suture-bone graft transplanted into an experimental unilateral premaxillo-maxillary bone and suture defect, Engdahl et al., 1978). These studies not only support the growth site theory of sutures, they also emphasize the adaptive nature of the suture to the milieu of forces in which it is placed and this forms the basis for the present investigation: that connective tissues of fibrous joints are responsive to mechanical stress.

Tooth eruption is also a controversial subject and a number of theories have been proposed (for review see Osborn and Ten Cate, 1983b). Both the periodontal ligament fibres and its associated fibroblasts have been implicated in tooth eruption. Yet,

whether the periodontal ligament plays a primary or secondary role is still a point for debate. The presence of myofibroblasts with contractile properties in the periodontal ligament space is still being examined.

Whether growth of the craniofacial complex and tooth eruption is intrinsic or reactive to environmental factors, adaptive remodelling activities of the osteocytic and fibrocytic series of cells have to be operative. In this respect, the two structures are remarkably similar. Events in rapidly growing interparietal suture of rats (Ten Cate et al., 1977), continuously erupting mouse incisors (Melcher, 1967) or reactivation of limited eruption in mouse molars (Melcher and Correia, 1971) all involve remodelling of soft connective tissues and bone.

However, there are differences in connective tissue biochemistry between the sutural and periodontal joints in terms of collagen phenotype composition and rate of turnover. Collagen is the most abundant structural protein of the body with a ubiquitous distribution. At present, at least five types of collagen are known and with different body distributions. Different types of collagen may co-exist in different proportions depending on the type of tissue and its physiologic state. The collagen of the periodontium and gingiva has long been a subject of interest because of its unique biochemistry, in particular of the high rate of turnover (Beertsen and Everts, 1977; Beertsen, 1979) and increased proportion of type III when compared with other tissues (Sodek, 1976; Limebach et al., 1978; Narayanan and Page, 1983a, 1983b).

Using [^{3}H]-proline radioautography, it has been shown that the periodontal ligament has a very high rate of collagen turnover under normal physiological conditions (Stallard, 1963; Carneiro and Fava de Moraes , 1965; Anderson, 1967; Skougaard et al., 1970; Kameyama, 1973, 1975). Biochemical studies have also indicated a high turnover rate for collagen in marmoset gingiva (Page and Ammons, 1974) and rat mandibular bone (Firschein, 1967). Sodek (1976) has shown directly that the collagen turnover in soft and mineralized connective tissues of rat molar periodontium is appreciably higher than skin which is itself high relative to other connective tissues (Kao et al., 1961). It was also found that the rate of collagen synthesis and the efficiency of conversion of newly synthesized collagen to mature collagen in the tissues of the periodontium is higher than gingiva, skin and bone. The half-life of mature collagen of the periodontal ligament was found to be 1 day, the gingiva 5 days, the alveolar process 6 days and the skin 15 days (Sodek, 1977). Similar studies for growing and non-growing sutures have yet to be done.

Except under specific circumstances, such as during fetal life (Epstein, 1974) and wound healing (Gay et al., 1978), type I and III collagens usually co-exist in tissues with type I predominating (Epstein and Munderloch, 1975). However, it has been demonstrated in vivo that although type I collagen is the major collagen in periodontal tissues, significant quantities of type III collagen are also present in bovine periodontal ligament (Butler et al., 1975), human gingiva (Ballard and Butler, 1974; Narayanan and Page, 1983a, 1983b) and bovine cementum (Birkedal-Hausen et

al., 1977). Similar findings in the mouse periodontium have also been demonstrated in vitro (Yen and Melcher, 1978). Both types of collagen with a similar high rate of turnover (Sodek, 1976, 1977; Sodek and Limebach, 1979).

Comparable information for sutures is beginning to be available. Chiang (1981), in an in vivo study of collagen phenotype synthesis by interparietal suture of adolescent mouse under mechanical stress, reported that type III collagen composed 6% of total collagen in sham-operated control animals. However, in a similar study by Meikle and co-workers (1982) using neonatal rabbits, but carried out in vitro, no evidence of type III was found in control animals. Pilot projects for the present investigation show the presence of type III collagen in rapidly growing interparietal suture of the mouse and will be discussed in detail later. Unfortunately, information about rate of collagen turnover in sutures is still lacking.

The reason for this relatively high proportion of type III collagen and rapid turnover of all collagens in periodontal tissues is obscure. The mechanical stress from intermittent occlusal forces which could produce microtrauma in the collagen fibres of these tissues, particularly during mastication, may be important (Sodek, 1976; Limeback et al., 1978). Thus, the periodontal tissues may have to be under constant repair even during normal physiological conditions. This is comparable with other situations with a high rate of synthetic and remodelling activities, such as when skin is undergoing rapid growth and development (Epstein, 1974), or during wound repair (Gay et al., 1978) and during in

vivo stretching of the interparietal suture of the adolescent mouse (Yen et al., 1980) or in vitro stretching of the interparietal suture of the neonatal rabbit (Meikle et al., 1982).

One of the common denominators of the above situations is the presence of a significant quantity of type III collagen, though its turnover rate is still unknown.

Thus, it can be argued that the phenotypic expression and high rate of turnover of collagen in the periodontal tissues is a reflection of the reaction of cellular components to local functional demands rather than an inherent difference in their biochemical behaviour.

(3) Response to Mechanical Stress

Craniofacial sutures are subjected to tensile stress generated during growth and development during oro-facial muscle activity and when adapting to spatial relationships of bones (Baer, 1954; Pritchard et al., 1956). The periodontal ligament has to accommodate tooth eruption and becomes stress-bearing once teeth are in functional occlusion. The capability to respond to mechanical stress is natural to the fibrous joints and is important for the process of growth and development of the animal. They both possess the identical microstructure and the identical remodelling response to mechanical stress, whether it is from growth and development, daily activities or orthodontic and facial orthopaedic therapy (Hinrichsen and Storey, 1968; Moffett, 1971, 1973; Storey, 1972).

Observations from the study of the non-human primates indicated that sutural joints are easily remodelled. Through cellular remodelling of these articular surfaces (Moffett, 1971, 1973), the growth occurring in the craniofacial sutures can be quantitatively altered or reversed from bone deposition to resorption and vice versa. This is very similar to cellular activity in the periodontal ligament when subjected to orthodontic tension and compression. Also, any orthodontic appliance which transmits force beyond the periodontal joints will produce similar remodelling activity in the facial and cranial sutures. This principle finds frequent orthodontic application in palatal suture expansion and when extra-oral appliances are used to displace the maxillary structures in a certain direction (Moffett, 1973). These joints are most easily remodelled because of their vascularized fibrous articular tissue. The ease with which the sutural and periodontal joints can be mechanically remodelled forms the basis of orthodontic and facial orthopaedic therapy for malocclusions and skeletal malformations, especially in young growing patients.

Tensile force applied to sutures and periodontal ligament evokes similar responses including cell proliferation (Taylor et al., 1968; Baumrind and Buch, 1970; Linge, 1972; Roberts and Jee, 1974; Yee et al., 1976; Gould et al., 1977; Meikle et al., 1980; Chiang, 1981), protein synthesis (Crumley, 1964; Diaz, 1978; Meikle et al., 1979, 1982; Chiang, 1981) and bone formation (Macapanpan et al., 1954; Waldo and Rothblatt, 1954; Zaki and Van Huysen, 1963).

Tissue changes occurring within the periodontal ligament

during orthodontic tooth movement have been well documented histologically (for review, see Reitan, 1975). The effects of mechanical forces on the craniofacial sutures have also been well described cephalometrically and histologically (Murray and Cleall, 1971; Moffett, 1971, 1973; Nelson, 1972; Badell, 1976; Kambara, 1977; Nanda, 1978; Jackson et al., 1979).

Sutural response to mechanical stress may seem complicated at the first glance. Nanda (1978) in an analysis of the zygomaticomaxillary suture relative to the protraction force system demonstrated a very complicated response as some areas of the suture were in compression while other areas were under tension. This was due to the existence of rotational components of the protraction force system and from the complicated three-dimensional anatomy of the suture. However, all areas of tension showed stretching of collagen fibres and eventual bone apposition, while areas of compression demonstrated disorientation of collagen fibres followed by osteoclastic resorption of bone surfaces. Also, a generalized increase in cell proliferation in sutures under tension paralleled the increase of the mitotic index in areas of the periodontal ligament under orthodontic tension (Taylor et al., 1968; Roberts and Jee, 1974; Nanda, 1978) or after wounding (Gould et al., 1977).

However, the most remarkable similarity between the two structures has to be in their capability to regenerate instead of forming scar tissue after injury or trauma (Gilman, 1968). This presumably is achieved by identical metabolic activities of the cellular component of both structures (Ten Cate et al., 1976,

1977). During rapid expansion of the rat interparietal suture, an initial inflammatory response was followed by a period of overwhelming fibrogenesis and osteogenesis with subsequent remodelling of bone and the suture by the osteocytic and fibrocytic cell series until normal sutural dimension and architecture were achieved (Ten Cate et al., 1977). This is remarkably similar to the cellular activities of the periodontal ligament during normal physiologic tooth movement (Ten Cate et al., 1976) and involves the same cell populations. Collagen profiles have been observed in macrophages during involution of the post-partum mouse uterus (Parakkal, 1969a) and have been associated with collagen turnover during the hair growth cycle (Parakkal, 1969b). Although macrophages are present in rat interparietal suture and periodontal ligament, collagen profile containing vesicles have never been found to be associated with them (Ten Cate et al., 1976, 1977; Yee, 1979). It seems that only the fibrocytic series of cells is responsible for collagen remodelling in sutures and periodontal ligament.

In addition, a parallel exists between the developing suture in the rapidly growing animal and the suture during rapid expansion (Ten Cate et al., 1977). After the acute inflammatory phase during expansion has subsided, presumably when the force created by the expansion spring diminishes to a threshold level (equalling the growth force), the same events occur in both situations, namely tremendous collagen deposition followed by remodelling by fibroblasts. Given that the fibroblasts of the periodontal ligaments also have a similar role of fibrogenesis followed by remo-

delling during orthodontic tooth movement, it should be possible to bring about an orderly remodelling of the periodontium if the "threshold force level" can be delivered by our appliances to the periodontium. Unfortunately, all the available evidence indicates that during tooth movement, even with light forces, 5 gm in rat and 70 gm in man (Buck and Church, 1972; Rygh, 1972, 1973) damage in the form of hyalinization, occurs to the periodontal ligament. Thus, orthodontic tooth movement, as practised today, is a pathologic process from which the tissue recovers with repair followed by regeneration. Most of the damaged material in an area of hyalinization has to be removed before repair can be initiated, resulting in delay of tooth movement (Skillen and Reitan, 1940).

The fact that a cellular mechanism exists within the periodontal ligament to permit an orderly remodelling during physiologic tooth movement suggests that this mechanism should be utilized to achieve therapeutic tooth movement (Ten Cate et al., 1976). What is required is information concerning the factors which control the ligament fibroblasts in their synthetic and degradative functions. One of the objectives of the present investigation is to try to shed some light into the differences in sutural response under tensile stress of different force levels.

In summary, the sutural and periodontal fibrous joints are comparable anatomic entities because they are structurally similar, especially in terms of functional cellular elements and, at least before full maturation, respond to mechanical stress in a similar fashion. Thus, both structures could serve as a model for the further study of fibrous joints in response to mechanical

stress and the choice depends purely on strategic considerations.

Cranial sutures, especially the interparietal suture (Hinrichsen and Storey, 1968; Ten Cate et al., 1977; Meikle et al., 1979, 1980, 1982; Yen et al., 1980; Yen and Suga, 1981; Chiang, 1981; Duncan, 1982; Yen and Chiang, in press) were often used in the past because of their greater accessibility and simple geometry and will serve as the experimental model for the present investigation as well.

Although caution must be exercised when extrapolating experimental data derived from cranial sutures to explain the dynamics of remodelling in the periodontal joints, the discussion in this chapter suggests this approach could be a viable and reasonable proposition.

(C) METHODOLOGY AND FINDINGS OF CHANGES OF FIBROUS JOINTS INCIDENTAL TO MECHANICAL STRESS

The capability of fibrous joints to respond to mechanical stress has long been exploited for orthodontic and facial orthopaedic purposes. The hard and soft connective tissue changes occurring within the periodontal ligament during orthodontic tooth movement have been well documented histologically, at both the light and the electron microscopy level (for review, see Reitan, 1975). Similarly, suture remodelling in response to orthopaedic stress has been studied histologically (Cleall et al., 1965; Murray and Cleall, 1971; Moffett, 1971, 1973; Droschl, 1975; Linge, 1972, 1976), and cephalometrically (Haas, 1970; Moffett, 1971, 1973). In addition, remodelling of sutures quite distant from the dento-alveolar complex resulting from posteriorly (Droschl, 1973; Elder and Tuenge, 1974) and anteriorly (Nanda, 1978) directed traction to the maxillary dentition has also been studied histologically and cephalometrically. Based upon this information, various "theories" of the biology of tooth movement were advanced, attempting to identify and explain the cellular mechanism responsible for the observed histological and morphological changes. However, all these "theories" have to offer so far is only a description of the histologically observed phenomena incidental to tooth movement plus some ingenious postulates and speculations. The biochemical pathway and the cellular control mechanisms of the periodontal ligament remodelling activity are still largely unknown. Nevertheless, these "theories" will be briefly reviewed to illustrate previous approaches and their ina-

dequacies.

(1) Pressure-Tension Theory

Originally presented by Sandstedt (1904, 1905a, 1905b) and later supported by Schwartz (1932) and Gianelly (1969), this hypothesis involved the concept of vascular occlusion causing the histologically observable events of hyalinization and undermining bone resorption. More recently, bone deformation (Baumrind, 1969) and thixotropic behaviour of the periodontal ligament (Kardos and Simpson, 1980) have been proposed as alternative interpretations of the pressure-tension theory.

(2) Oxygen-Tension Theory

Bien (1966) hypothesized that capillaries constricted by compression of periodontal ligament fibres form a series of cirsoid aneurysms. Below each site of stenosis, decreased blood pressure results in formation of minute oxygen bubbles which can diffuse through the vessel wall and lodge against bone surfaces leading to osseous resorption.

(3) Piezo-Electric Theory

It has been observed that electric potentials are generated by the application of force to bone (Fukada and Yusada, 1957) including the alveolar bone (Zengo et al., 1973, 1974). An

increase in electrical activity has been associated with an increase in osteogenic activity, as demonstrated by elevations in osteoblast number and quantity of bone formation (Bassett et al., 1964; Bassett, 1968; Zengo et al., 1976), as well as a corresponding increase in intracellular activity (Davidovitch et al., 1978a, 1978b, 1979, 1980a, 1980b).

Even after the resolution of whether the pressure-tension theory is due to vascular occlusion, bone deformation or thixotropic behaviour of the periodontal ligament or maybe a combination of all three, it still does not offer an explanation of the cellular mechanisms involved. Similarly, an increase in osteogenic cell number and intracellular activity have been associated with piezo-electric potentials as a result of mechanical deformation of the dento-alveolar complex. Yet the method of transduction of the electric current into the observed cellular behaviour has not been delineated, although hypotheses involving the migration of electrons through biologic membranes (Jahn, 1962) proton charge transport through the cytoplasm (Eigen and DeMaeyer, 1958) and amphoteric ion exchanges (Jahn, 1968) have been advanced. Relationship between oxygen tension and bone resorption has been demonstrated in vitro (Goldhaber, 1958, 1961, 1966; Stern et al., 1966), but no attempts have been made to either measure fluctuations in oxygen tension or to manipulate oxygen tension within the periodontal ligament and observe the effects upon bone resorption. Although increased vascularity of the periodontal ligament has been associated with frontal bone resorption occurring with the application of orthodontic force (Gianelly, 1969; Khouw and

Goldhaber, 1970), the question of whether the increased vascularity found in areas of active bone resorption is primary or secondary to the resorption has yet to be resolved. Thus, the inadequacies of all the three classical theories of orthodontic tooth movement are evident.

This is why orthodontics and facial orthopaedics as practised today are still rather intuitively and empirically based; more of an art than a science. We still do not have the answers to fundamental questions such as:

(a) What is the optimal force level such that remodelling is the most efficient with the shortest treatment time and yet, the least treatment sequelae if any?

(b) What is the ideal reactivation schedule?

(c) Are there different optimal force levels and different ideal reactivation schedules depending upon the type of movement required and the particular fibrous joint(s) needed to be remodelled?

(d) Is continuous activation always better than intermittent activation?

(e) How do we define intermittent activation?

(f) Are there treatment modalities other than that of mechanical?

(g) What is the potential for pharmacological agents in the practice of the future?

(h) What effects would combined mechanical and pharmacological agents have on treatment response?

The answers to the above and many other similar questoins

depend on the elucidation and understanding of the biochemical pathway and their control mechanism of connective tissue remodelling and would allow us to have a better understanding and realization of the scope and limitation of orthodontics and facial orthopaedics. This basic understanding is paramount if orthodontics and facial orthopaedics are to be practised rationally and scientifically based; thus maximizing treatment efficiency and stability while minimizing treatment sequelae and relapse potential. The knowledge would also give us a better understanding of the disease states when fibrous joints behave abnormally, e.g. in Apert's, Crouzon and Treacher Collins Syndromes, in cleidocranial dysostosis and in tooth ankylosis. In addition, the etiology of malocclusion as well as dysplastic craniofacial skeletal relationship of fibrous joints could be better understood which may lead to their prevention and/or treatment.

Recently, the direction of research has been more on the cellular and molecular levels of the biochemical aspect of orthodontic tooth movement. Hormones and other physiological mediators with known effects on the metabolism of osseous tissues have been studied in relation to orthodontic tooth movement. In particular, the ability of parathyroid hormone to enhance orthodontic tooth movement, both micro- and macroscopically, has been recorded by Gianelly and Schmur (1969), Kamata (1972) and Davidovitch and co-workers (1972).

Davidovitch and co-workers (1975, 1976a, 1976b, 1977) have also associated fluctuations in cyclic nucleotide levels with areas of tension and compression in periodontal ligament of cats

following tooth movement *in vivo*.

In addition, the possible role of prostaglandins as a mediator of bone resorption during orthodontic tooth movement was first investigated by Yamasaki and co-workers (1980) in cats.

However, the lack of localized specificity of the parathyroid hormone presents technical difficulties in the study of its possible involvement in the regulatory mechanism of orthodontic tooth movement. Similarly, it is difficult to assess the specific role of cAMP which functions as a secondary messenger in almost all aspects of physiology of both high and low forms of life (Rasmussen, 1970). Although prostaglandins have been implicated in both physiological and pathological bone and collagen metabolism (for review, see Duncan, 1982), and also have been shown to be produced in response to mechanical stretching of cells *in vitro* (Binderman and Cox, 1977; Somjen *et al.*, 1980), their role in fibrous joint remodelling is still quite controversial (Duncan, 1982).

Thus, findings from the above studies are usually inconclusive and generally, the methodology employed is not amenable to expansion and development for further investigation.

Moreover, these "fishing expedition" approaches tend to generate quantities of data that are often difficult, if not impossible, to relate with one another and generally only reveal phenomenon of association rather than provide cause and effect information, and are thus of limited value.

A more rational and potentially fruitful approach would be first to identify the cellular and extracellular components that

are actively involved in fibrous joint remodelling during orthodontics and facial orthopaedics, followed by the development of a model system which can be precisely defined and also readily reproduced. The elucidation of the sequence of events on the cellular and molecular levels of the biochemical aspect of fibrous joint remodelling in response to mechanical stress can then be approached systematically using the model system developed and may eventually lead to the understanding of the regulatory and control mechanisms.

Collagen is the most abundant structural protein of fibrous joints (Barbanell et al., 1978) and could be most significant in light of its contribution to the unique architecture and function of these tissues (Page and Schoeder, 1982). The most notable ultra-structural characteristic of fibroblasts and osteoblasts in tension zones of rat molar periodontium incidental to orthodontic tooth movement is a highly developed system of rough endoplasmic reticulum, suggesting a high level of matrix synthesis by these cells of which collagen is the main component (Rygh, 1976). Electron microscopic studies also indicated that remodelling of the periodontal ligament during normal physiologic tooth movement and when cranial sutures are subjected to tensile mechanical stress involves rapid turnover of collagen macromolecules (Ten Cate et al., 1976, 1977).

Diaz (1978) indicated a decrease in collagen degradation in his study of the effect of applied force in the periodontal ligament of the rat using [^{3}H]-proline radioautography, while Meikle and co-workers (1979) reported a two-fold increase in protein and

collagen synthesis in expanded cranial sutures of neonatal rabbits *in vitro*.

Thus, not only is collagen the major structural component of fibrous joints, but its metabolism is also central to their remodelling activity. The study of collagen biochemistry in fibrous joints during normal growth and development, as well as when subjected to therapeutic mechanical manipulation, may provide insight into the regulators and control mechanisms of the remodelling process.

(D) BIOCHEMISTRY OF COLLAGEN

(1) Structure and Biosynthesis

Collagen is the main extracellular component of all connective tissues (Bailey and Robins, 1976; Kivirikko and Risteli, 1976; Bornstein and Traub, 1979). It is the most abundant structural protein and represents about one-quarter to one-third by weight of the total body protein. It is synthesized primarily by mesenchymal cells such as fibroblasts, chondroblasts, osteoblasts and odontoblasts. Basement membrane protein, however, is secreted by epithelial cells (Barbanell et al., 1978).

Collagen is synthesized following the normal path of protein synthesis with unique post-translational modifications in the primary structure of the molecule (Kivirikko and Risteli, 1976; Barbanell et al., 1978; Minor, 1980). This includes hydroxylation of proline and lysine residuals, glycosylation of some of the hydroxylysine residuals, pro-alpha chain association, intra- and inter-chain bond formation, as well as the formation of the triple helical structure. Collagen is secreted as procollagen, the soluble or secretory form of collagen, which has additional terminal peptides. Procollagen is converted to tropocollagen with the enzymatic cleavage of a small polypeptide from the N-telopeptide portion of the procollagen molecule. Tropocollagen is insoluble and represents the simplest building block unit of collagen in the extracellular space. Tropocollagen molecules spontaneously aggregate into fibrils by formation of both intra- and inter-molecular crosslinks (Tanzer, 1973). Further aggregation of collagen

fibrils give rise to collagen fibres (Barbanell et al., 1978; Prockop et al., 1979a, 1979b).

A single collagen molecule is about 15 Å in diameter and 2800 Å in length with a molecular weight of about 300,000. This is the tropocollagen molecule and consists of three separate polypeptide chains, termed alpha chains, intertwined into a right-handed, rod-like triple helix with each alpha chain coiled in a left-handed helix of its own.

Each alpha chain consists of about 1052 amino-acid residues and the primary structure of each is essentially of the repeating triplet (gly-X-Y)n which consists of glycine and two other amino acids. The Y position is often occupied by proline or hydroxyproline. Hydroxyproline is found almost exclusively in collagen and changes in hydroxyproline contents are interpreted as representing changes in collagen metabolism. The other constituent amino acids most commonly occupy the X position. Thus, about one-third of the amino acids are accounted for by glycine, while proline and hydroxyproline together comprise about one-quarter of the amino acids in each alpha chain. Alanine, a neutral amino acid, makes up about 9%. Lysine and hydroxylysine, which are important in the formation of the inter-molecular linkages, and hydroxylysine is also the site of glycosylation, constitute only about 1.3% of the total amino acids in the molecule.

However, collagen is not a homogeneous protein, probably because the functional requirements for collagen in different connective tissues may not be the same and cannot be fulfilled by a single type of molecule.

The collagens represent a group of at least five closely related but genetically distinct structural proteins designated as type I, II, III, IV and V (Bornstein and Sage, 1980; Minor, 1980; Prockop and Williams, 1982). Their distribution and relative amount are tissue specific though it appears that almost all tissues contain more than one type of collagen. Also very few, if any, of the collagen types have a unique distribution and it is likely that as methods of detection become more sensitive, a broad distribution range will be found for each of the collagens (Bornstein and Sage, 1980).

The different collagen types can be separated by chromatography and electrophoresis and each collagen phenotype can be characterized and identified by its own cyanobromide digest peptide pattern (Bornstein and Sage, 1980).

(2) Distribution and Physiology

Type I collagen is the most abundant species. It is the only type found so far in the mineralized connective tissues and predominates in skin, tendon, bone, dentin, gingiva and the periodontal ligament. It is composed of two identical and a third structurally different alpha chains, with the molecular formula of [alpha 1 (I)]2 alpha 2 (I).

Type II collagen seems to be cartilage specific (Miller, 1972; Bornstein and Sage, 1980; Minor, 1980). It is composed of three identical alpha chains with the molecular formula of [alpha 1 (II)]3.

Type III collagen, as [alpha 1 (III)]3, seems to co-exist with type I, but in different proportions. Moreover, the relative proportion can change with age, e.g. skin (Epstein, 1974); with the type of tissue, e.g. gingiva and the periodontal ligament (Butler et al., 1975; Sodek and Limeback, 1979); with altered metabolic states, e.g. diseased gingiva (Narayanan et al., 1980, 1983; Narayanan and Page, 1983a), and granulation tissues during wound healing (Miller, 1976; Gay et al., 1978). Type III is also present in blood vessels (Bornstein and Sage, 1980).

Type IV collagen, as [alpha 1 (IV)]3 is the major collagen type in basement membranes (Bornstein and Sage, 1980).

Type V collagen was first isolated from fetal membranes (Burgeson et al., 1976). However, later studies revealed that it is present, as a minor component, along with type I and III or type II (Rhodes and Miller, 1978) in most connective tissues, and that it has a unique ultrastructural localization in pericellular spaces and near basement membranes (Madri and Furthmayr, 1979; Roll et al., 1980).

The alpha chain composition of type V collagen is [alpha 1 (V)]2 alpha 2 (V) (Rhodes and Miller, 1978). Alpha 1 (V), alpha 2 (V) and alpha 3 (V) chains represent B, A and C chains of the old nomenclature respectively (Bornstein and Sage, 1980). Though alpha 3 (V) chain described in the gingival tissue by Narayanan and co-workers in 1980 is a contaminant and not a constituent of type V (Narayanan and Page, 1983b).

In addition, type I trimer with the molecular formula of [alpha 1 (I)]3 are synthesized under diseased or artificial condi-

tions. It can be found in diseased gingiva (Narayanan and Page, 1983a) as well as skin, cartilage, aged chondrocytes and fibroblasts cultured in vitro (Minor, 1980). It is also synthesized in organ cultures of rat incisor odontoblasts (Munksgaard et al., 1978). In in vitro culture of mouse periodontium explants with low PO2 (100-120 mm Hg), the ratio between alpha 1 (I) and alpha 2 (I) chains was found to be as high as 6:1 suggesting synthesis of large amount of type I trimers (Yen, 1978). Although it seemed that type I trimers are usually synthesized under abnormal or altered physiological and/or biochemical conditions only, it has also been found in chick embryo tendons and calvaria in vivo (Jiminez et al., 1977). Type V can also exist in the molecular form of [alpha 1 (V)]3 (Rhodes and Miller, 1978).

Not only is collagen the main structural protein in connective tissues, histological (Stallard, 1963; Carneiro and Leblond, 1966) and biochemical (Sodek, 1977; Sodek et al., 1977) studies have demonstrated that collagen is active metabolically as well. This metabolic activity, often in response to the physiological and functional demands of the particular connective tissue, often resulted in a change of the amount and relative proportions of the different collagen phenotypes, especially for types I and III, which usually co-exist in the same tissues.

It has been suggested that a high proportion of type III collagen often associates with situations when rapid collagen synthesis is required. Thus, while type I is predominant in adult skin, type III accounts for over 60% of the total collagen in fetal skin (Epstein, 1974; Chung and Miller, 1974; Miller, 1976). Also type

III accounts for 15% of the total collagen in the rat periodontal ligament (Sodek and Limeback, 1979) and 20% in the bovine periodontal ligament (Butler et al., 1975). This could be a reflection of the more severe and constant physical and bacterial stresses placed on the periodontium which may be equivalent to a state of continuous wound healing and repair (Claycomb et al., 1967). Biochemical studies (Page and Ammons, 1974; Sodek, 1976; Sodek and Limeback, 1979) have also shown that in comparison with that in other connective tissues, the turnover of collagen in the periodontal ligament is rapid. The reasons for this are not clear, but it has been suggested that intermittent occlusal forces acting on the collagen fibres, particularly during mastication, may be of relevance (Sodek, 1976).

The relative proportions of type I and III collagen are also being affected in various abnormal and pathological states. Proportions of type III are increased during inflammation (Weiss et al., 1975), in early phases of wound healing in skin (Gay et al., 1978) and in matrix-induced osteogenesis (Reddi et al., 1977). Type III collagen persists in the dentin of patients with osteogenesis imperfecta (Penttinnen et al., 1975; Sauk et al., 1980) and is synthesized by synovial cells from patients with rheumatoid arthritis (Eyre and Muir, 1975), while it is deficient in patients with hereditary Ehlers-Danlos Syndrome type IV (Pope et al., 1975; Aumailley et al., 1980).

Thus, not only do quantitative differences in the proportions of the different collagen types in local anatomic sites clearly exist and appear related to normal tissue function, but modulated

synthesis of the several collagens may also be crucial for the normal progression of dynamic processes such as development and tissue repair (Bornstein and Sage, 1980). Failure of the mechanism often results in various disease states.

(3) Collagen Phenotype Ratio as a Biological Signal

Collagen is the principal structural protein of fibrous joints. Its integrity is essential for normal development and function of this connective tissue entity. One would expect that under similar conditions, the metabolic behaviour of collagen in fibrous joints would be the same as that in other connective tissues. Orthodontic tooth movement has been likened to an inflammatory wound healing response (Storey, 1973; Ten Cate et al., 1976) similar to other situations with rapid collagen synthesis and remodelling and where the proportion of type III/type I + III is significantly affected. Thus, collagen phenotype ratio can serve as an important parameter, indicating the metabolic state of the fibrous joints during the remodelling process in response to orthopaedic and orthodontic stress. This change in collagen phenotype ratio can serve as an indicator for the change in cellular activities and for the study of the regulation of collagen synthesis which is crucial to the understanding of fibrous joint remodelling during growth and development and when subjected to therapeutic manipulation.

(E) IN VITRO ORGAN CULTURE OF MOUSE INTERPARIETAL SUTURE AND THE STUDY OF FIBROUS JOINTS

(1) Necessity for an In Vitro Organ Culture System

Tissue remodelling of fibrous joints occurs during craniofacial growth and tooth eruption, establishment and maintenance of dental occlusion, orthopaedic manipulation of developing craniofacial structures and during orthodontic tooth movement. However, the mechanism of control of fibrous joint remodelling in response to mechanical deformation is largely unknown despite the many theories proposed: the pressure-tension theory, the oxygen-tension theory, the piezo-electric theory, and the theory of metabolic mediators which include parathyroid hormone, cAMP, prostaglandins and others. To confirm or refute any of the above propositions requires unravelling of the cellular events incidental to fibrous joint remodelling. Over the years, numerous attempts have been made to clarify the role of mechanical force in the remodelling of fibrous joints. In general, these investigations were mostly limited to histological and morphological methods of analysis as investigations of the factors regulating cellular activity are difficult to control in in vivo experiments. Contributions from these studies are mainly in the correlation of therapeutic mechanism with treatment results, while the cellular events and their regulating mechanisms that lead to the histological and morphological changes are still essentially largely unknown.

It is unlikely that the validity of any of the proposed theo-

ries or the direct role of any regulators can be established by experiments *in vivo*, because of the difficulty of controlling the numerous variables that are present in whole animal experimental systems. Also, for the study of the effects of hormones and other metabolic mediators, particularly those with generalized effects to the whole animal, *in vivo* studies cannot differentiate the direct from the indirect effects of mediators on the remodelling fibrous joint. In addition, biochemical and molecular experimentation often requires the use of radioactive isotopes and an *in vitro* system is often necessary because of the often prohibitive cost of doing *in vivo* isotope experiments. An *in vitro* system in the form of an organ culture, therefore, seems likely to provide the most promising experimental model for testing theories proposed and specifying biochemical pathways and the role of regulators in fibrous joint remodelling.

(2) Development of In Vitro Organ Culture Systems of Dental Tissues

In vitro organ culture of dental tissues was pioneered by Glasstone and contemporaries in the 1930's (for review, see Yen, 1978). These early studies helped to define the essential elements and the basic techniques for maintaining viability and continuing morphogenesis of tooth germ organ cultures *in vitro*.

Initially, natural media consisted of chick plasma and embryo extracts, in the form of a clot, were used for *in vitro* culture of molar tooth germs from rat and rabbit fetuses. Explant viability

was maintained and normal cusp pattern developed in tooth germs that had been explanted before the cusps appeared (Glasstone, 1936, 1938, 1952, 1954). Also, it was demonstrated that explants cultured "on the clot" exhibit greater development than explants cultured "in the clot" (Lefkowitz and Swayne, 1956a). These experiments indicated that:

(a) both nutrient availability and gaseous exchange are important for cellular viability and differentiation, and

(b) morphogenetic regulators are retained in the explants, suggesting control is local at or after the stage of growth and development when tooth germs were explanted.

Attempts were also made to maintain explants in fluid media, consisting mainly of serum and embryo extracts, for the enhanced availability of nutrients in the medium to the explants and the fascilitation of analysis. It was found that periodic gassing with an oxygen rich gaseous mixture (Szabo, 1954) or maintaining the explant at the gas-medium interphase with support provided by filter membranes (Koch, 1965, 1967) or by using the hanging-drop technique (Hay, 1961) was necessary for continuing viability of explants. This further substantiates the importance of gaseous exchange in _in vitro_ organ culture systems.

One of the major drawbacks of using natural media for _in vitro_ culture studies is the impossibility to totally define their constituents qualitatively and/or quantitatively, which are essential for studies of regulators and control mechanisms of metabolic processes. However, attempts to maintain tooth germs in chemi-

cally defined media proved unsuccessful initially, when rat tooth germs were cultured "in" chemically defined fluid medium (Lefkowitz and Swayne, 1956b, 1958). Glasstone (1964) used mouse molar explants cultured "on" lens paper rafts floating on chemically defined protein-free fluid medium. Explants differentiated but failed to form enamel matrix. The success of Glasstone's system was attributed to the smaller size of the mouse molar explant and to the efficient gaseous exchange when the explant was situated at the gas-medium interphase. Using the culture system originally developed by Trowell (1959), in which explants were cultured on filter membranes supported by stainless steel grids on the surfaces of fluid media, Thesleff (1976) demonstrated mouse odontoblast would differentiate in chemically defined medium though serum extract was essential for matrix secretion by ameloblasts. Trowell (1954, 1959) also found that a gas mixture of 95% oxygen and 5% carbon dioxide was essential for in vitro culture of mature tissues. Thus it appeared that successful in vitro organ culture depends upon the efficient delivery of nutrients and oxygen to the cellular elements of the explant. The accessibility of explants to an adequate supply of oxygen has played an important role in determining the design of the various systems of organ culture in vitro. At present, for short term in vitro culture of explants of limited size, the Trowell-type culture system (Trowell, 1959) is one of the most efficient and economical systems available, especially when radioisotopes are involved because of the small amount of culture medium used in such a system.

Long term in vitro culture of mouse single-molar and

periodontium explant was successful using a glass circulator system (Turnbull and Melcher, 1974; Melcher and Turnbull, 1976), while mouse three-molar and periodontium explant was also successful but necessitated the use of a continuous flow culture system (Yen and Melcher, 1978). However, the Trowell-type culture system with serum-free medium and a continuous flow of 95% O2 and 5% CO2 gas mixture proved, histologically and radioautographically, to be adequate for 24-hour culture of a mouse explant of three molars and periodontium (Duncan, 1982), as well as mouse calvaria explants (Yen and Suga, 1981; Duncan, 1982) with no signs of oxygen toxicity. This seemed to provide the best in vitro organ culture system for the study of fibrous joints.

(3) Mouse Interparietal Suture as a Model for the Study of Fibrous Joints

Modern day orthodontics and craniofacial orthopaedics involve remodelling of periodontal and sutural joints in response to therapeutic mechanical manipulation and the effects of mechanical stress on both anatomic structures should be investigated. However, the close resemblance of the sutural and periodontal fibrous joints suggests that the findings from the study of one structure may be extrapolated to that of the other. The preference of one structure to the other in the design of an animal experimental model depends largely upon which can better fulfill the experimental objectives with as simple a design as possible.

As a large number of animals would be required for the pres-

ent investigation, the mouse is chosen because of its small size and a large number can be bred and maintained easily and economically.

Although the technical difficulties of isolating and maintaining in culture an explant composed of a single mouse molar with its periodontium have largely been resolved (Turnbull and Melcher, 1974; Melcher and Turnbull, 1976) and studies using mouse three-molar explants have also been successful (Yen and Melcher, 1978; Duncan, 1982), it is still difficult to deliver force of known magnitude to dental units of small experimental animals like the mouse. Also, intra-oral appliances invariably affect food intake, especially immediately after the placement of the appliance. Presumably, this is a period with active, remodelling activity and good nutritional supply would be of utmost importance. This is because when tissues are being repaired, more amino acids, carbohydrates, lipids, minerals, water and oxygen are consumed than during catabolism and anabolism of normal mature tissue (Pollack, 1979).

The mouse interparietal suture is readily accessible with a mid-line incision of the scalp and the calvaria is large enough to accommodate the implantation of the helical spring that delivers the mechanical stress to the suture. The simple geometry which fascilitates definition of force direction and the reasonable quantity of tissue available all indicate that the interparietal suture would serve as the ideal site for study. Also, explants with the interparietal suture can be easily dissected for <u>in vitro</u> culture studies with minimal disturbance to the suture. The geom-

etry of the explant with its large surface area to volume ratio ensures easy accessibility of medium and gaseous exchange with the Trowell-type culture system and makes studies requiring radioisotopes possible because of the small amount of medium used in such a system. The choice of male mice only for the present study is arbitrary, to eliminate variables due to sexual dimorphism, especially those dependent on the stage of growth and development.

It has been suggested previously that investigations of factors regulating cellular activity is difficult using in vivo experimentation. It appears that a model system in which fibrous tissue and bone can be maintained in vitro is essential if progress is to be made towards understanding the molecular control mechanisms involved in fibrous joint remodelling. However, for all in vitro organ culture systems, there is a time limit that an explant can be maintained viable, and more importantly, still retain the in vivo biologic behaviour, such that findings and conclusions from in vitro experimentation can be extrapolated to in vivo situations. Meikle and co-workers (1979) reported an organ culture system whereby mechanical stress can be applied to a fibrous joint under controlled conditions. This produces an experimental model that would mimic the continuous force to which the periodontal and other sutural joints are exposed during orthodontic and craniofacial orthopaedic treatment. However, the maximum experimental period was limited to four days as histological and biochemical criteria suggested some cellular degradation when explants were cultured beyond five days. No doubt, the in vitro culture time limit can be greatly extended, albeit still finite,

using more sophisticated culture systems like the continuous flow culture system (Yen and Melcher, 1978). However, such systems are usually quite involved and not suitable for investigations involving a large number of experimental animals. Moreover, when radioisotopes are required, the cost can be prohibitive. A better system would be whereby quantified mechanical stress can be applied to cranial sutures for various periods of time in vivo followed by in vitro pulse labelling of organ explants under controlled experimental conditions. Thus, only a relatively short period of in vitro organ culture is required. This allows the use of the simple Trowell-type culture system and would be equally suitable for the study of long term effects of mechanical stress to fibrous joints, as the in vitro explant organ culture period will always be the same and of short duration, equals the pulse labelling period. The short labelling period also eliminates the complicating factor of amino acid recycling which can be up to 93% efficient as found in guinea pig skin recycling of proline (Jackson and Heininger, 1975). Such a system of in vivo stress followed by in vitro pulse labelling used in the present investigation, may yet prove to be best able to reflect the in vivo biological events because of the short period of time that the explant has to be removed from its natural environment.

CHAPTER II

STATEMENT OF THE PROBLEM

Collagen metabolism is central to fibrous joint remodelling during growth and development and when physically stressed. However, the behaviour of collagen during remodelling is largely unknown. The identification of an appropriate parameter that would indicate the metabolic state and change in cellular activity of the remodelling fibrous joints may elucidate such behaviour. Knowledge of this behaviour would be the first step in identifying effects of control mechanisms and ultimately of the control mechanisms themselves which regulate fibrous joint remodelling. The eventual clinical manipulation of such control mechanisms would facilitate more efficient and less traumatic anatomical remodelling as required in orthodontic therapy. The first experiment will ascertain whether the proportion of type III collagen may serve as such an indicator. The interparietal suture of mouse was selected because of its capacity to respond in earlier *in vivo* remodelling studies (Yen *et al.*, 1980).

Although *in vitro* systems are better suited than those *in vivo* for the study of factors regulating cellular activity, including collagen metabolism, there will be a time limit that an organ culture can be maintained viable and more importantly, still retain comparable *in vivo* metabolic activities. The second experiment will demonstrate the suitability of the *in vitro* model system developed for the study of newly synthesized type III collagen in fibrous joints undergoing rapid remodelling.

The third experiment will determine the ratio of type III collagen during normal growth and development of mouse interparietal suture. This will establish base-line data representing rapid

collagen remodelling under normal physiological conditions and will determine the age of the mouse when the suture would be metabolically stable and suitable for orthopaedic experimentations.

Lastly, as extrinsic mechanical manipulation is the only treatment modality for malocclusion and skeletal disharmonies, the sutural response due to tensile stress of five different force levels will be studied and compared with that during rapid growth and development under normal physiological conditions. This may provide insight in the concept of "optimal force" level (relative to collagen synthesis) such that therapeutic objectives can be achieved efficiently and without iatrogenic effects.

CHAPTER III

EFFECT OF TENSILE FORCE ON COLLAGEN PHENOTYPE SYNTHESIS IN MOUSE INTERPARIETAL SUTURE *IN VIVO*

SUMMARY

The ratio of type III collagen to the total of type I and type III collagens in force-stressed mouse interparietal sutural tissue was determined at various times after force application. Ten 9 week old Swiss male white mice had helical springs placed surgically in their calvaria to expand the interparietal sutures. Ten control mice were sham-operated without force application. Two stressed mice and two non-stressed mice were sacrificed at each of five time intervals: 12 hours, 3 days, 7 days, 10 days and 14 days, and their interparietal sutural tissues dissected for collagen extraction in neutral salt solutions. Using SDS-polyacrylamide gel electrophoresis, it was found that the stressed sutures contained a significantly ($P < 0.05$) higher ratio of type III collagen relative to the non-stressed sutures for the periods of 12 hours to 7 days after force-application. By 10 days there was no significant difference between stressed or non-stressed sutures. This pattern parallels findings in wound-healing and matrix-induced bone formation and suggest a role for type III collagen in the initial stages of remodelling. The higher ratios of type III found in periodontal ligament indicate lower remodelling activity in sutures, possibly due to less mechanical stress.

INTRODUCTION

The craniofacial sutures comprise those soft and hard connective tissues which interact at the margins of craniofacial intramembranous bone. The capacity for these tissues to remodel is essential in the normal growth and development of the craniofacial skeleton and in the gross morphological response required in the clinical correction of skeletal disproportions when the periodontal ligament and sutures are subjected to orthodontic and facial orthopaedic forces. While there have been several gross morphological and histological descriptions of sutural remodelling activity, both during normal growth (Cleall et al., 1971; Persson, 1973) and in response to orthopaedic force (Cleall et al., 1965; Brossman et al., 1973; Linge, 1976; Ten Cate et al., 1977), the mechanisms which regulate the synthesis and resorption of these tissues are still unknown. Since collagen is the predominant protein in the sutural tissues (Persson, 1973; Ten Cate et al., 1977), monitoring of collagen activity should reflect the remodelling. A short term study by Meikle et al. (1982) has indicated the appearance of type III collagen in rabbit sutures expanded in vivo but labelled in vitro, while finding no type III collagen in unstressed sutures. The aim of this study was to determine the pattern of collagen phenotype synthesis found at longer periods of time in interparietal sutures of adolescent Swiss male white mice after expansion with helical springs compared to sham-operated controls.

MATERIALS AND METHODS

Surgical Technique

Swiss male white mice, 9 weeks old, inbred in our facility were divided into 5 sacrifice groups: 12 hrs, 3, 7, 10 and 14 days, each group having 2 control and 2 experimental animals. Surgery was performed under sodium pentobarbital anaesthesia (Nembutal, Abbott Laboratories, Montreal, Quebec), 0.07 mg/g body weight plus a local injection of xylocaine (2% xylocaine, 1:50,000 epinephrine, Astra Laboratories, Mississauga, Ontario) underneath the scalp. A midsagittal incision was made through the scalp exposing the calvaria. Holes were drilled through the calvaria 2 mm on either side of the interparietal suture, using a 1/4 round burr mounted in a highspeed handpiece. Helical expansion springs were pre-formed by means of a template, for a consistent force level of 50 g $\pm$ 5% when activated by 2.0 mm, from 0.016" Tru-Chrome orthodontic wire (Rocky Mountain/Orthodontics, Denver, Colorado) and inserted into the holes. The skin flaps were reapposed and the incision closed with 4-0 silk sutures (Ethicon, Peterborough, Ontario). Sham-operated animals minus springs served as controls.

At each of the post-operative times the 2 control and 2 experimental animals were sacrificed by cervical dislocation and the interparietal sutural tissues carefully dissected out, placed in 1.5 ml plastic tubes and frozen.

Salt-Extraction of Collagen

The frozen tissues were thawed and treated as follows: extracted for 27 hrs on a rotating platform at 4°C (3 X 9 hrs) in 3 X 500 ul of 50 mM tris-HCl, pH 7.4, 0.45 M NaCl, 20 mM EDTA, 1 mM hydroxymercuribenzoate, 10 um phenylmethylsulfonyl fluoride and 0.005% bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri). The neutral salt supernatants were pooled and dialyzed against the extraction buffer for 6 hrs, followed by dialysis for 16 hrs against 1% HAc. The samples were frozen and lyophilized. The suture residues were acid extracted for 4 hrs at 4°C with 0.5 N HAc and then digested twice with pepsin (0.1 mg/ml in 0.5 N HAc; Sigma Chemical Co., St. Louis, Missouri) at 16°C for 10 hrs each time. All samples were frozen and lyophilized.

Electrophoresis

Aliquots of the lyophilized samples were subjected to SDS/polyacrylamide slab gel electrophoresis using a modified method of Laemmli (1970) and utilizing the interruption method of Sykes et al. (1976) to achieve a separation of type I and type III collagen alpha-chains. Control samples used duplicate aliquots that were incubated with purified bacterial collagenase (1 mg/ml, Collagenase, Cl. histolyticum CLSP, Worthington Biochemical Corp., Freehold, New Jersey). Other duplicate aliquots were electrophoresed without the presence of beta-mercaptoethanol. The

gels were stained with Coomassie Blue using the technique of Fairbanks et al. (1971) and scanned and calculated on a Beckman DU-8 Spectrophotometer with Gel Accessory (Beckman Instruments, Palo Alto, California).

RESULTS

Both type I and type III collagen (identified with standards) were demonstrated for all salt-extracted samples, stressed and non-stressed. Aliquots incubated with bacterial collagenase failed to demonstrate bands in the positions expected for types I and III alpha-chains. Aliquots not treated with beta-mercaptoethanol failed to show any migration of type III alpha-chains. The ratio of type III alpha-chains relative to total collagen bands is summarized in Table III-1. Using t-tests, ratios of type III collagen were significantly higher ($P < 0.05$) at 12 hrs, 3 days and 7 days in the stressed sutures relative to the non-stressed sutures.

Analysis of variance did not indicate a significant difference between the mean values of ratios of type III collagen for the stressed sutures over the time period studied. However, it did indicate a significant change ($P < 0.05$) for ratios of type III collagen in the non-stressed sutures, and a multiple comparison test (Newman-Keuls) indicated that the mean value for percentage of type III at day 14 in non-stressed sutures was significantly higher ($P < 0.05$) than values in non-stressed sutures at earlier dates.

TABLE III-1

PROPORTION OF TYPE III α-CHAINS RELATIVE TO THE TOTAL OF TYPE I AND TYPE III α-CHAINS AS DETERMINED FROM DENSITOMETRIC SCANS OF COLLAGEN BAND SEPARATED BY SDS-PAGE AND STAINED WITH COOMASSIE BLUE

	Duration of Spring Implant				
	12 hr	3 day	7 day	10 day	14 day
Stressed Sutures %	13.9±3.9 *	19.4±0.9 *	15.4±1.1 *	12.0±0.9	11.6±2.5
Non-Stressed Sutures %	7.7±0.8	7.8±1.0	8.1±1.0	8.4±1.0	13.4±0.1

N = 2 for each value which represent mean ± 1 standard deviation.

* $P < 0.05$

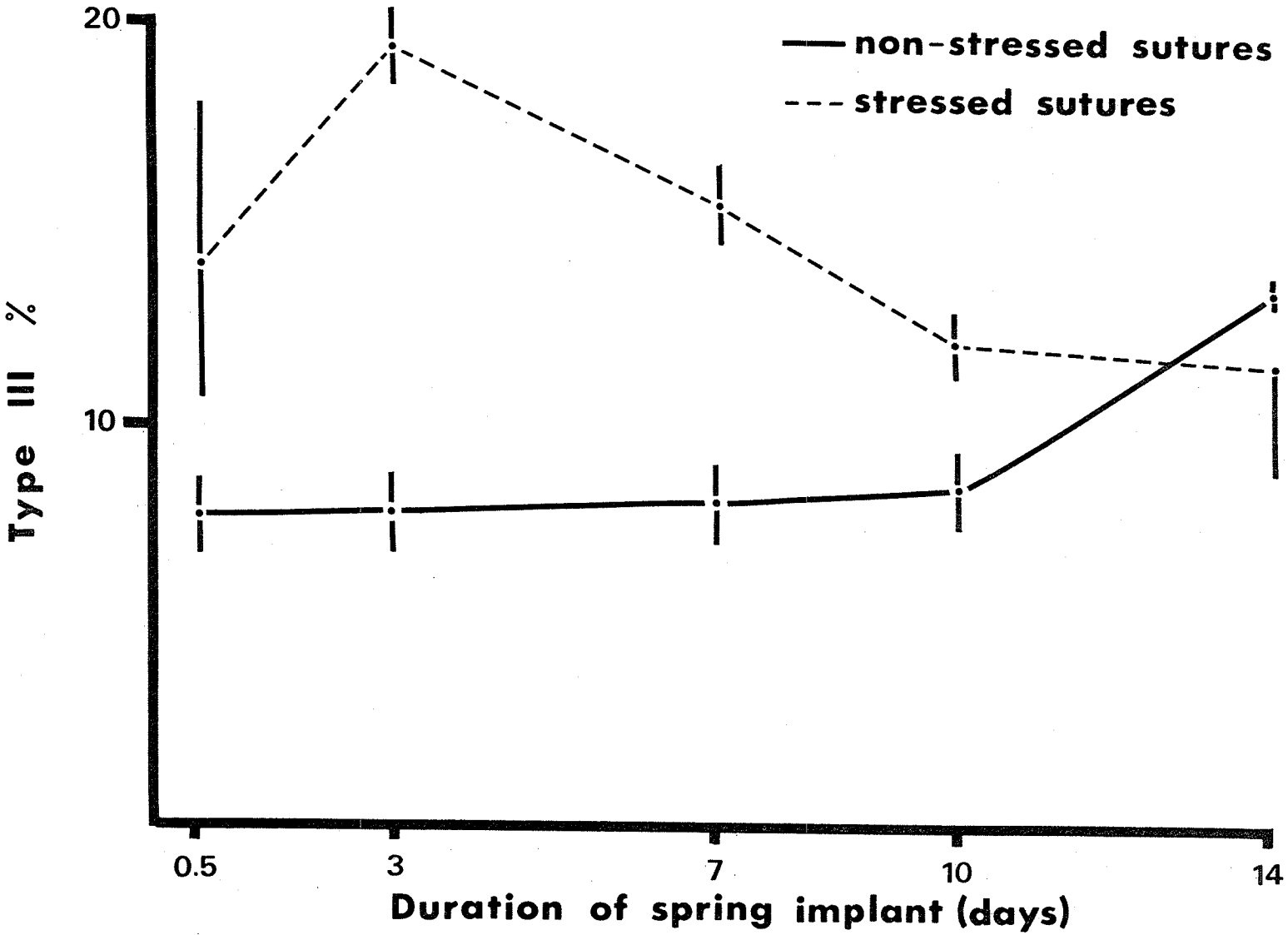

Fig. III-1 PROPORTION OF TYPE III vs. DURATION OF IMPLANT

DISCUSSION

The significantly higher ratios of type III in mechanically stressed sutures at time points up to and including 7 days agrees with the work of Meikle et al. (1982) who found increases in ratios of type III in newly-synthesized collagen at 12 hrs and 96 hrs. Because this study looked at total salt-soluble collagen, ratios of type III in stressed sutures are lower than those reported by Meikle et al. (1982) who were measuring only newly-synthesized collagen. Interestingly, Meikle et al. (1982) could not detect any type III in untreated control sutures. One would expect from rapidly growing tissues high proportions of type III collagen (Epstein, 1974). The body weights of the rabbits used by Meikle et al. (1982) were within the 150-200 g range and most likely were very young rabbits undergoing rapid growth and development, especially in the bony cranium. The subject will be discussed further in Chapter V.

The non-stressed sutures in this study were probably affected by the sham-operation, even though no mechanical force was applied, and thus demonstrated about 7% ratio of type III until 14 days after spring application when inexplicably the value increased to stressed-suture ranges of 13%. Without further time points and a much bigger sample size, it is difficult to explain the significance of this value. However, this aberrant result did not occur in subsequent experiments (Chapter VI) and likely be an artefact in the present case. Passive springs were not inserted into sham-operated controls due to the inability to secure them to

the calvaria. Generally, the values of the non-stressed sutures were much lower than the 15% found in rat periodontal ligament (Butler et al., 1975), despite the histological similarities between periodontal ligament and sutures. This would suggest that the sutural tissue may be subjected to different demands than those of periodontal tissue. Whether this is due to the more severe constant physical and bacterial stresses placed on the periodontium is unknown.

It appears that whenever there is a necessity for rapid extracellular matrix production, e.g. in embryonic tissues (Epstein, 1974); wound healing (Barnes et al., 1976; Gay et al., 1978) matrix-induced mesenchymal cell proliferation (Steinmann and Reddi, 1980) and presently with mechanically stressed sutures, the proportion of type III collagen is elevated. Also, the appearance of type III collagen during the early phases of normal healing (Gay et al., 1978) and of matrix-induced bone formation (Reddi et al., 1977) has been demonstrated by immunofluorescence. Although the functional significance of such a response is still unknown, the dynamics of collagen type III to total collagen ratio may serve as an important parameter, indicating the metabolic state of fibrous joints during the remodelling process in response to mechanical stress. This change in collagen phenotype ratio can serve as a signal for the change in cellular activities and for the study of the regulation of collagen synthesis. This may be crucial to the understanding of fibrous joint remodelling during growth and development and when subjected to therapeutic manipulation.

CONCLUSIONS

1). Mechanical tensile stress of predetermined force level can be delivered to the muose interparietal suture with implanted helical springs. The springs were well tolerated by the experimental animals with no incidence of infection.

2). Mechanically stressed mouse interparietal sutures contained a significantly ($P < 0.05$) higher ratio of type III collagen relative to the non-stressed sham-operated sutures from the periods of 12 hrs to 7 days after force application.

3). The pattern parallels findings in other systems with rapid extracellular matrix production and suggest a role for type III collagen in the initial stages of fibrous joint remodelling in response to mechanical stress.

4). The dynamics of collagen type III to collagen type I and III ratio may serve as a signal, indicating the metabolic state of fibrous joint remodelling in response to mechanical stress.

CHAPTER IV

COLLAGEN PHENOTYPE SYNTHESIS IN MOUSE INTERPARIETAL SUTURE UNDER TENSILE STRESS: AN *IN VIVO*, *IN VITRO* COMPARISON

SUMMARY

The ratio of type III collagen to the total of type I and type III collagens in force-stressed mouse interparietal sutural tissues in calvaria explant cutures was compared with that of *in vivo*. Eleven Swiss male white mice (9 weeks old) had helical springs placed surgically in their calvaria to expand the interparietal sutures. Four days after surgery, calvaria with the implanted springs were dissected from nine of the eleven mice and the explants cultured in Trowell-type organ culture dishes. [^{14}C]-glycine was added in triplicate, either immediately, or 60 minutes, or 120 minutes after the beginning of culture and all cultures were then continued for 2 hours after the addition of isotopes. The remaining two mice were sacrificed two hours after an intraperitoneal injection of [^{14}C]-glycine. The interparietal tissues were dissected for collagen extraction by limited pepsin digestion. Using SDS-polyacrylamide gel electrophoresis and fluorographic visualization of separated radio-labelled collagen bands, no significant difference in the ratio of type III collagen to the total of type I and type III collagens was found between *in vivo* and *in vitro* samples. Thus, during the short (four hours) culturing period, *in vitro* metabolic behaviour of calvaria explant is comparable with that *in vivo*.

INTRODUCTION

The capacity of fibrous joints to respond to mechanical stress has long been exploited for orthodontic and facial orthopaedic purposes. The hard and soft connective tissue changes in periodontal ligament during orthodontic tooth movement have been well documented histologically, at both the light and the electron microscopy level (for review, see Reitan, 1975). Similarly, sutural remodelling in response to orthopaedic stress has been studied histologically (Cleall et al., 1965; Murray and Cleall, 1971; Moffett, 1971, 1973; Droschl, 1973, 1975; Elder and Fuenge, 1974; Linge, 1976; Nanda, 1978) and cephalometrically (Haas, 1970; Moffett, 1971, 1973; Droschl, 1973, 1975; Elder and Fuenge, 1974; Nanda, 1978).

The theories of the biology of tooth movement: the pressure-tension theory (Sandstedt, 1904, 1905a, 1905b; Schwartz, 1932; Gianelly, 1969; Baumrind, 1969; Kardos and Simpson, 1980); the oxygen-tension theory (Goldhaber, 1958, 1961, 1966; Bien, 1966; Stern et al., 1966; Gianelly, 1969; Khouw and Goldhaber, 1970) and the piezo-electric theory (Fukada and Yusada, 1957; Eigen and Demaeyer, 1958; Jahn, 1962, 1968; Bassett et al., 1964; Bassett, 1968; Zengo et al., 1973, 1974, 1976; Davidovitch et al., 1978a, 1979, 1980a, 1980b) developed with information derived from these investigations, have proved to be inadequate in attempting to identify and explain the cellular control mechanisms of fibrous joint remodelling. Also, the biochemical pathway responsible for the observed morphological and histological changes are still

largely unknown.

Recently, hormones and other physiological mediators, e.g. parathyroid hormone (Gianelly and Schmur, 1969; Davidovitch et al., 1972; Kamata, 1972); c-AMP (Davidovitch et al., 1975, 1976a, 1976b, 1977) and prostaglandins (Yamasaki et al., 1980; Duncan et al., 1984), with known effects on the metabolism of osseous tissues have been studied in relation to orthodontic tooth movement. However, investigations of the factors regulating cellular activity is difficult using in vivo models. It is unlikely that the validity of any of the proposed theories or the direct role of any regulators can be established by experiments in vivo, because of the difficulty of controlling the numerous variables that are present in whole animals. Also, for studies of the effects of hormones and other metabolic mediators, particularly those with generalized effects to the whole animal, in vivo studies cannot differentiate changes due to the direct from those due to the indirect effects of mediators on the remodelling fibrous joints. In addition, biochemical and molecular experimentation often requires the use of radioactive isotopes and an in vivo system would limit their use due to the prohibitive cost. An in vitro system in the form of an organ culture, therefore, seems likely to provide the most promising experimental model for testing theories proposed and elucidation of biochemical pathways and roles of regulators involved in fibrous joint remodelling.

For any in vitro experimental model, however, one must be cognizant of whether in vitro explant cultures retain the in vivo biologic behaviour, such that findings and consequently conclu-

sions from *in vitro* experimentation can be extrapolated to *in vivo* situations.

The objective of the present investigation is to compare the *in vitro* remodelling activity of mechanically stressed mouse interparietal suture in calvaria organ explant with that of *in vivo*, as represented by the ratio of newly synthesized collagen phenotypes.

MATERIALS AND METHODS

Surgical Technique

Eleven 9 week old Swiss male white mice, imbred in our facility, had surgery performed under sodium pentobarbital anaesthesia (Nembutal, Abbott Laboratories, Montreal, Quebec) 0.07 ug/gm body weight plus a local injection of xylocaine (2% xylocaine, 1:50,000 epinephrine, Astra Laboratories, Mississauga, Ontario) underneath the scalp. A midsagittal incision was made through the scalp exposing the calvaria. Holes were drilled through the calvaria, 2.0 mm on either side of the interparietal suture, using a 1/4 round burr (Jet carbide burrs, Beavers Dental Products Ltd., Morrisburg, Ontario), mounted in a highspeed handpiece. Helical expansion springs were pre-formed by means of a template, for a consistent force level of 50 g $\pm$ 5% when activated by 2.0 mm, from 0.016" Tru-chrome orthodontic wire (Rocky Mountain/Orthodontics, Denver, Colorado) and inserted in the holes. The skin flaps were reapposed and the incision closed with 4-0 black silk suture (Ethicon, Peterborough, Ontario).

Dissection and In Vitro Calvaria Organ Explant Culture System

Four days after surgery, nine of the eleven mice were killed by cervical dislocation. Calvaria with the implanted springs were dissected from the mice and any opposing soft tissues were carefully removed. Using sharp dissecting scissors, each calvarium

was trimmed to a size of about 10 X 12 mm with the interparietal suture in the longitudinal midline of the explant.

The explants were placed on stainless steel grids (Falcon #3014, Becton, Dickinson and Co., Oxnard, California) at the gas (mixture of 95% 02 and 5% C02 humidified by bubbling through distilled water) to the medium (Waymouth's 752/1, Grand Island Biological Co., Grand Island, New York) interphase in Trowell-type (Trowell, 1959) organ culture dishes (60 X 15 mm, Falcon #3037, Becton, Dickinson and Co., Cockeysville, Maryland) containing 800 ul of supportive medium (Waymouth, 1959) supplemented with 300 ugm/ml ascorbic acid, 330 U/ml penicillin, 0.75 ugm/ml amphotericin B and 330 ugm/ml streptomycin. The centre wells of the dishes were surrounded by absorbent filter paper rings moistened with sterile distilled water. The dishes were covered and the explants were cultured at 37°C in a humidified incubator.

Twenty uCi per ml [^{14}C]-glycine (Amersham Corporation, Oakville, Ontario) with a specific activity of 52.2 mCi/mmol was added to three sets of three culture dishes at 0, 60 and 120 minutes after the beginning of the explant culture. All explant cultures were continued for 120 minutes after the addition of isotopes and the interparietal sutural areas (Moss, 1954) were then carefully dissected out. Each sample was placed in 1.5 ml plastic tubes and frozen after washing twice with 0.5 ml of fresh culture medium to remove free isotopes.

The remaining two animals were given intraperiteneal injections of [^{14}C]-glycine (1.0 mCi/100 gm body weight) and killed two hours post injection. The interparietal sutural areas were care-

fully dissected out, placed in 1.5 ml plastic tubes and frozen similarly.

Pepsin Extraction of Radio-labelled Collagen

The frozen tissues were thawed and subjected to limited pepsin digestion for collagen solubilization. Each sample was digested three times with 500 ul of pepsin (2 X crystallized, Sigma Chemical Co., St. Louis, Missouri) solution (0.1 mg/ml in 0.5 N acetic acid) at 16°C for 12 hours. After each digestion, the samples were centrifuged at 10,000 X g for 3 minutes and the supernatants were removed and frozen. The residues were resuspended in 500 ul of fresh pepsin solution for further digestion. After removal of supernatants of the third digestion, the residues were washed by resuspension in 500 ul of 0.5 N acetic acid, centrifuged and the washings removed. The supernatants and the washing of each sample (total volume 2.0 ml) were pooled and mixed thoroughly. 100 ul of each sample were placed in miniscintillation vials with 5 ml of scintillation cocktail (Aquasol, New England Nuclear, Boston, Massachusetts) and counted for [^{14}C] in a liquid scintillation counter (Nuclear Chicago Isocap 300, Searle Instrumentation, Toronto, Ontario). The remaining sample was frozen and lyophilized in preparation for gel electrophoresis.

Collagen Separation and Quantification

Collagen and procollagen alpha-chains were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis on 20 cm slab gels. The procedure followed for the separation of those collagen components was described by Laemmli (1970) and utilized a 7.5% cross-linked separating gel, a 2.5% stacking gel and Tris/glycine buffers (Bio-Rad Laboratories, Richmond, California). Type I and type III collagen alpha-chains were separated using the interrupted electrophoresis method of Sykes and co-workers (1976).

Freeze-dried samples were dissolved in 70 ul of reservoir buffer containing 2M urea, 2% sodium dodecyl sulphate and 0.1% bromophenol blue and heated at 60°C for 30 minutes to denature the collagen. Samples were introduced to the sample wells and electrophoresis was performed for one hour at 130 V. Electrophoresis was stopped and samples were then reduced by the addition of 20% mercaptoethanol to the sample wells to allow type III alpha-chains to penetrate the gel. After standing for 60 minutes, electrophoresis was continued until the tracking dye reached the base of the gel.

For fluorographic visualization of separated radio-labelled collagen bands, gels were washed twice in dimethyl sulfoxide (Fisher Scientific, Fair Lawn, New Jersey) and impregnated with 2,5-diphenyloxazole (New England Nuclear, Boston, Massachusetts) as described by Bonner and Laskey (1974). The gels were placed on filter paper and dried on a slab drier (Bio-Rad, Richmond, California) and exposed to Kodak XRP-1 x-ray films (Kodak Canada

Inc., Toronto, Ontario) at -60°C for varying periods of time as indicated by the scintillation counts of the samples.

Individual sample tracks from the developed fluorographs were then scanned at 550 nm and proportions of type III collagen relative to the total of type I and type III collagens calculated on a spectrophotometer (Beckman DU-8, Toronto, Ontario).

RESULTS

Type I and type III collagens with a high proportion of type III were demonstrated in all *in vivo* and *in vitro* samples. The ratios of type III alpha-chains relative to the total of type I and type III alpha-chains are summarized in Table IV-1. Using t-tests, no significant difference was found between the ratios of type III collagen from the three *in vitro* conditions and that of *in vivo*.

TABLE IV-1

PROPORTION OF TYPE III α-CHAINS TO THE TOTAL OF TYPE I AND TYPE III α-CHAINS

	III / I + III %	Average % ± S.D.
In Vivo	20.85; 11.22	16.03 ± 6.81
In Vitro (0 min.)	13.00; 19.55; 16.48	16.34 ± 3.28
In Vitro (60 min.)	19.19; 10.94; 20.53	16.89 ± 5.20
In Vitro (120 min.)	14.38; 20.67; 17.54	17.53 ± 3.15

In Vitro (0 min.) = [^{14}C]-glycine added at the beginning of calvaria explant culture and continued for 2 hours.

In Vitro (60 min.) = [^{14}C]-glycine added at 60 minutes after the beginning of calvaria explant culture and then continued for 2 hours.

In Vitro (120 min.) = [^{14}C]-glycine added at 120 minutes after the beginning of calvaria explant culture and then continued for 2 hours.

DISCUSSION

Articular remodelling is of fundamental importance in the clinical practice of orthodontics and facial orthopaedics. Nevertheless, little is known of the response of fibrous joints to mechanical deformation at the molecular level. Previous studies were mostly limited to morphological and histological methods of analysis. This is because investigation of the factors regulating cellular activity during remodelling of fibrous joints consequent to mechanical stress is difficult using *in vivo* experimentation. It seems that a model system in which fibrous tissue and bone could be maintained *in vitro* will be essential if progress were to be made towards understanding the fundamental mechanisms involved. However, *in vitro* systems of analysis also have their limitations. There will be a time limit that an *in vitro* organ culture can be maintained viable and, more importantly, still retain the *in vivo* biological activities such that results and conclusions from *in vitro* experimentations can be extrapolated to *in vivo* situations. In addition, it would be ideal if the *in vitro* system can be precisely defined and readily reproduced. Also, it should be economical even if a large number of experimental animals and isotopes have to be used.

Previous studies (Szabo, 1954; Trowell, 1954, 1959; Lefkowitz and Swayne, 1956b, 1958; Hay, 1961; Glasstone, 1964; Koch, 1965, 1967; Thesleff, 1976; Kollar, 1980, 1981) indicated that successful *in vitro* organ culture depends upon the efficient delivery of nutrients and oxygen to the cellular elements of the explant. The

accessibility of explants to an adequate supply of oxygen has played an important role in determining the design of the various systems of organ culture in vitro.

Although long term in vitro organ culture was successful with the glass circulator system (Turnbull and Melcher, 1974; Melcher and Turnbull, 1976) and the continuous flow culture system (Yen and Melcher, 1978), these complicated systems are not suitable for investigations when large numbers of experimental animals and isotopes are likely to be involved.

In vitro organ explant culture systems of simpler design where fibrous joints can be stressed and then radio-labelled for study were reported by Meikle and co-workers (1979) and by Hickory (1982). However, the maximum experimental period in both cases were limited as signs of cellular degeneration were noted after five days and as early as 28 hours, respectively. Thus, it becomes clear that a simple and economical in vitro organ culture system that is also suitable for the study of long term effects of mechanical stress to fibrous joints is urgently needed.

At present, for short term in vitro culture of explants of limited size, the Trowell-type culture system is one of the most efficient and economical systems available. Not only does it provide the important gas-medium interphase essential for in vitro culture of mature tissues, but it also is particularly suitable for experimentation when radioisotopes are involved, because of the small amount of culture medium used in such a system. To overcome the limited in vitro culturing period, quantified mechanical forces can be applied to cranial sutures for various periods

of time *in vivo* followed by *in vitro* pulse labelling of organ explants under controlled experimental conditions. This system is equally suitable for the study of long term effects of mechanical stress to fibrous joints, as the *in vitro* organ explant culturing period will always be of the same short duration, that of the pulse labelling period. This short period of *in vitro* culture needed in the present system has many other advantages as well.

Firstly, the probability that the explant retains its *in vivo* biologic properites and behaviour will be enhanced because of the very short duration that the explant has to be removed from its natural environment.

Secondly, the short labelling period of two hours used in the present case will allow for maximal incorporation of isotope with minimal time for degradation of newly synthesized collagen (Sodek, 1976, 1977). It also eliminates the complicating factor of amino acid recycling, which can be up to 93% efficient as found in guinea pig skin recycling of proline (Jackson and Heininger, 1975).

Thirdly, vitamin C, which would oxidize rapidly in the medium when exposed to light, oxygen and incubation temperature (Feng *et al.*, 1977), can be minimized. An optimal concentration of vitamin C is essential for collagen synthesis (Waerhaug, 1958; Yen, 1978; Sodek *et al.*, 1982).

In order that the *in vitro* organ explant culture system can be precisely defined and readily reproduced, only serum-free synthetic culture medium can be used, because of the undefined complex composition of serum extracts. Yet, the studies of regula-

tors and control mechanisms of metabolic processes often require the knowledge and the monitoring of the qualitative and quantitative changes in the properties of the constituents of the culture medium. In addition, serum extract may have a direct effect of its own upon the metabolic behaviour of the organ explant. Serum has been shown to have a small stimulating effect on growth rate and protein synthesis (Nolan et al., 1978). The presence of serum also has a direct influence on collagen metabolism. Moreover, the effect of serum on different collagen phenotypes are different. Synthesis of type I collagen increases significantly, but only slightly for type III (Narayanan and Page, 1977).

Finally, in vitro organ explant culture system is most suitable for the study of the direct effects of hormones and other metabolic mediators, as effects due to a systemic response of the organism will be eliminated.

In vitro explant culture of mouse three molar periodontium (Duncan, 1982), and mouse calvaria (Yen and Suga, 1981) were reported, using Trowell-type culture system with serum-free meidum and a continuous flow of humidified 95% O2 and 5% CO2. Tissue viability after 24 hours was demonstrated, both histologically and radioautographically, using [^{3}H]-proline and [^{3}H]-thymidine labelling.

However, the surgical procedure of explantation and the impossibility of simulating exactly the in vivo situations by an in vitro culture system may affect tissue behaviour, even though cellular viability may have been maintained. For example, the differentiated state of fibroblasts, with respect to collagen

synthesis, appears to be rigidly controlled under conditions of the extracellular environment (Hance and Crystal, 1977). Results obtained from in vitro studies are very often dependent upon the conditions of the tissue culture system (Muller et al., 1975; Abe et al., 1979). Therefore, it is important to compare the in vitro metabolic behaviour of the organ explant with that of in vivo for assessing the validity of using it as a model system for further investigations.

Using a continuous flow organ culture system, Yen (1978) reported that the pattern of collagen synthesis by periodontal explants in vitro is comparable to the in vivo pattern. Similar studies of in vitro organ explants, using the much simpler Trowell-type culture system, demonstrated a comparable pattern of collagen to non-collagenous protein synthesis ratios (Yen and Suga, 1981) between in vivo and in vitro suture. However, no comparison has been made with regards to ratio of newly synthesized collagen phenotypes. It has been shown that the ratio of newly synthesized type III collagen to the total of type I and type III collagens is very sensitive to the changes in the extracellular environment (Hance and Crystal, 1977), and is chosen as the parameter for comparison (Chapter III).

[^{14}C]-glycine was used for labelling because of the predominant proportion of glycine relative to the other amino acids in collagen ensuring maximum radioactivity in the newly synthesized collagen. In addition, a more linear relationship between fluorographic response and radioactivity of radio-labelled protein bands would be obtained (Bonner and Laskey, 1974).

The limiting factor in the methodology used in the present investigation lies in the extraction of collagen by pepsin digestion. For the correlation of the measured ratio with that in the original tissue to be valid, collagen in the pepsin digest must be representative of the total population of collagen molecules. The likelihood for this being so increases as a greater proportion of the collagen is brought into solution by pepsin. Results obtained by solubilization of 70%-80% of human skin collagen were reported to be comparable with data obtained by cyanogen bromide digestion, which effectively extract over 95% of the collagen (Sykes et al., 1976). The present model only requires the efficient extraction of the newly synthesized radio-labelled collagen molecules. These newly synthesized collagen with only limited number of intra- and intermolecular cross-links would be easily solubilized with pepsin digestion. Thus, the ratio obtained would likely be the true representation of that in the original tissue.

The present investigation demonstrated that phenotype expression of newly synthesized collagen under the three in vitro situations are all comparable to the in vivo pattern. More importantly, the in vitro culture with isotopes added at the beginning of the culturing period indicated a fast recovery of in vivo metabolic behaviour from the surgical procedure of explantation and the sudden change from in vivo to in vitro environment. In fact, this may not seem so surprising as explantation was done carefully with cutting edges as far removed from the interparietal suture as possible. The explant was then positioned at the gas-medium interphase for culture immediately. Thus, most, if not all, of

the immediate micro-environment of the sutural tissue was maintained with minimal disturbance. Also, the *in vivo* metabolic behaviour was continued *in vitro* for at least four hours, the maximum culturing period.

This *in vivo* stress followed by *in vitro* pulse labelling with the simple Trowell-type culturing system may yet prove to be best able to reflect the *in vivo* biological events, because of the short duration that the explant has to be removed from its natural environment. Such a system would have considerable potential for the study of regulators and control mechanisms of fibrous joint remodelling due to growth and development or when artificially stressed. The findings of which would have direct consequence to the clinical practice of orthodontics and facial orthopaedics.

CONCLUSIONS

1). A model system in which mouse calvaria can be stressed *in vivo*, then labelled *in vitro* using the Trowell-type culture system with chemically defined, serum-free medium and under controlled experimental conditions has been developed.

2). *In vitro* metabolic behaviour of calvaria explant was found to be comparable with that *in vivo* as indicated by the phenotype ratio of newly synthesized collagen for a period of four hours.

3). The system is equally suitable for the elucidation of short as well as long term effects of parameters under study; as the *in vitro* organ explant culturing period will always be the same short duration, that of the pulse labelling period.

4). The simple design of the *in vitro* culture system makes it possible for investigations that require large numbers of experimental animals and the use of isotopes.

5). The system would have considerable potential for the clarification of the biochemical pathway and the study of regulators and control mechanisms of fibrous joint remodelling during growth and development and when artificially stressed. The findings of which would have direct consequence to the clinical practice of orthodontics and facial orthopaedics.

CHAPTER V

COLLAGEN PHENOTYPE SYNTHESIS IN MOUSE INTERPARIETAL SUTURE DURING GROWTH AND DEVELOPMENT *IN VITRO*

SUMMARY

The ratio of collagen type III to the total of type I and type III in mouse interparietal sutural tissues was determined at selected ages from newborn to adulthood (36 weeks old). Using the *in vitro* radio-labelled culture system, mouse calvaria with expansion at the interparietal suture were dissected and the explants cultured in Trowell-type organ culture dishes. [^{14}C]-glycine was added sixty minutes after the beginning of culture and all cultures were then continued for two hours after the addition of isotopes. The interparietal sutural tissues were dissected for collagen extraction by limited pepsin digestion. Using SDS-polyacrylamide gel electrophoresis and fluorographic visualization of separated radio-labelled collagens, the ratio of collagen type III alpha-chains to the total of type I and type III alpha-chains was found to be age dependent. The proportion of type III alpha-chains at birth was quite high, followed by a significant* drop during the first two days of life, probably due to the sudden change from *in utero* to animal room environment. The proportion of type III alpha-chains rose significantly* from day 2 to day 4, reaching a maximum and then dropped significantly* to about the same proportion as at birth by day 7. There was a further significant* drop during the second week of life with the proportion stabilized at around 3.5% from 2 weeks to 10 weeks of age. A final significant* drop during the eleventh week of life

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Note: *Significant changes are at $P < 0.05$.

led to no detectable type III collagen after 12 weeks old. A correlation between the changes in the collagen type III ratio in the mouse interparietal suture and the histological changes in the rat interparietal suture from newborn to adulthood was attempted. The changes in the collagen phenotype ratio does not seem to relate to changes in body weight during growth and development, suggesting the interparietal suture may have an independent maturing pattern. The desirable age for future orthopaedic studies in Swiss male white mouse interparietal suture would be between 9 and 10 weeks of age.

INTRODUCTION

The capacity of fibrous joints to remodel is essential for the normal growth and development of the craniofacial skeleton and the development and maintenance of functional occlusion. There is a continuing change of relationship between the tooth and its supporting structures during tooth eruption and between the bones and articulations of the craniofacial skeleton during growth and development. Also, the mechanical stress to the sutural and periodontal fibrous joints during muscle activities of the face as well as during functional and parafunctional activities of the dentition require the integrity of this remodelling process of fibrous joints be maintained throughout life.

With the exception of the mandible, the clinical practice of orthodontics and facial orthopaedics is primarily the controlled mechanical manipulation of sutural and periodontal fibrous joints via the same remodelling process. Thus, orthodontics and facial orthopaedics can be essentially visualized as an accelerated version of this normal process of biological adaptation, termed remodelling such that a significant amount of remodelling has to be accomplished in a relatively short period of time. The ultimate clinical aim would be to effect this remodelling process via the most efficient and least traumatic pathway possible.

The first step towards this ultimate goal should be the establishment of baseline data beginning with the understanding of fibrous joint behaviour under normal physiological conditions. Thus, effects of different treatment modalities or variables of a

treatment modality can be compared and evaluated. Also, this may help us to have a better understanding of the disease states when fibrous joints behave abnormally, e.g. premature synostosed sutures in Apert's, Crouzon and Treacher Collin Syndromes; failure or delay in suture fusion and tooth eruption of cleidocranial dysostosis patients and abnormally fused periodontal ligament in tooth ankylosis. The etiology and thus the possibility of prevention and treatment of these conditions depend on the understanding of the biology of fibrous joints.

Collagen is the main extracellular component of all connective tissues (Kivirikko and Risteli, 1976; Bornstein and Traub, 1979). However, collagen is not a homogeneous protein and the distribution and relative amount of the different collagen types are tissue specific. There are quantitative differences in the proportions of the different collagen types in local anatomic sites and are important for normal tissue function. Also, modulated synthesis of the several collagens may be crucial for the normal progression of dynamic processes such as development and tissue repair (Bornstein and Sage, 1980). Thus, changes in the collagen phenotype ratio, especially that of type I and type III, may serve as an important parameter indicating the metabolic state of the connective tissue involved and may act as a signal for the changing in cellular activities (Chung and Miller, 1974; Epstein, 1974; Butler et al., 1975; Weiss et al., 1975; Miller, 1976; Reddi et al., 1977; Gay et al., 1978).

Collagen is also the most abundant structural protein of fibrous joints (Barbanell et al., 1978) and could be significant in

light of its contribution to the unique architecture and function of these tissues (Page and Schoeder, 1982). A change in collagen phenotype synthesis, with an increase in the type III ratio, to mechanical stress was demonstrated in the interparietal suture of adolescent mouse (Yen et al., 1980) and neonatal rabbit (Meikle et al., 1982). This is similar to other situations where rapid collagen synthesis and remodelling is the predominant phenomenon such as in fetal skin growth (Chung and Miller, 1974; Epstein, 1974; Miller, 1976), early phases of wound healing (Miller, 1976; Gay et al., 1978), inflammation (Weiss et al., 1975) and matrix-induced osteogenesis (Reddi et al., 1977). With electron microscopy, Ten Cate and co-workers (1977) have demonstrated that collagen metabolism is essential to fibrous joint remodelling during growth and development and when artificially stressed. Moreover, after the acute inflammatory phase has subsided in the case of extrinsic mechanical stress, the event occurring in both situations are remarkably similar, namely considerable collagen deposition followed by remodelling by fibroblasts.

However, the biochemical nature of fibrous joint collagen metabolism in response to therapeutic mechanical stress has never been compared with that during rapid growth and development. One of the objectives of the present study was to determine the nature of collagen phenotype synthesis in mouse interparietal suture from newborn to maturity, against which the effects of extrinsic mechanical stress can then be compared (Chapter VI). Also, for investigations of sutural response to mechanical stimulation, the induced effects have to be differentiated from the normal growth

and remodelling activity in the suture (Linge, 1972). Sutures of immature animals would risk masking any subtle change in the remodelling patterns by the high background of rapid growth. On the other hand, mature sutures in non-growing animals may not respond physiologically or not respond at all to orthopaedic force. It is important to determine the age of the laboratory animal when the suture would be metabolically stable, while retaining the cellular activity required for the potential to respond (Melcher, 1976; Hall, 1978a). In addition, a suture approximating this behavioural pattern would be most analogous to the mature functioning craniofacial suture encountered in the orthopaedic correction of skeletal disproportions as seen in orthodontic malocclusions.

Although the calvarial suture of various laboratory animals have been used for the study of fibrous joint remodelling during orthopaedic stress, including guinea pigs (Hinrichsen and Storey, 1968), rats (Ten Cate et al., 1977), mice (Yen et al., 1980; Yen et al., 1984) and neonatal rabbits (Meikle et al., 1979, 1980, 1982). However, much of the growth studies has been directed to the rat calvarium with no unanimity as to its age of maturity. Methodology used includes vital staining (Massler and Schour, 1951), anthropometry (Moss, 1954), single-dose radioisotope labelling (Young, 1962), multiple-dose radioisotope labelling (De Angelis, 1968) and electron microscopy (Ten Cate et al., 1977). So far, there are no comparable data for mouse calvarium. Mice would serve as a superior model for analysis of collagen and other connective tissue molecular activity, since the smaller body weight would be more economical for extensive radioisotope studies

and the smaller anatomical proportions offer greater potential for success in maintaining mature functioning sutures in vitro. At present, there are two studies about the growth and development of the mouse. General skeletal development was studied by Tonna (1965) using tritiated glycine radioautographic grain count data of the femur. The maturation of the interparietal suture was also studied radioautographically (Yen and Chiang, in press). There are no maturation studies of mouse cranial sutures with reference to the biochemical nature of collagen metabolism. The present investigation is for the study of collagen biochemistry during maturation of the mouse interparietal suture with reference to the ratio of collagen type III to the total of type I and type III. This will determine the range of this ratio occurring naturally in the mouse interparietal suture during rapid growth and development and the optimal age for studies of sutural remodelling in response to orthopaedic force.

MATERIALS AND METHODS

Dissection and In Vitro Calvaria Organ Explant Culture System

Swiss male white mice, inbred in our facility, were maintained on Wayne Laboratory, Blox-F6 specialty feed, given water ad libitum, and kept at a temperature of 70° ± 3°F. Six mice were randomly selected for each of the following age groups: newborn, 1 day, 2, 3, 4, 5, 6, 7, 10 days and three mice were randomly selected for each of the following ages: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 28, 32, 36 weeks. The body weights were recorded and mice were then killed by cervical dislocation. Calvaria, with the interparietal suture in the longitudinal midline, were dissected from the mice and any opposing soft tissues were carefully removed.

The explants were placed on stainless steel grids (Falcon #3014, Becton, Dickinson and Co., Oxnard, California) at the gas (mixture of 95% 02 and 5% C02 humidified by bubbling through distilled water) to medium (Waymouth's 752/1, Grand Island Biological Co., Grand Island, New York) interphase in Trowell-type (Trowell, 1959) organ culture dishes (60 x 16 mm, Falcon #3037, Becton, Dickinson and Co., Cockeysville, Maryland) containing 800 ul of supportive medium (Waymouth, 1959) supplemented with 300 ugm/ml ascorbic acid, 330 U/ml penicillin, 0.75 ugm/ml amphotericin B and 330 ugm/ml streptomycin. The centre wells of the dishes were surrounded by absorbent filter-paper rings moistened with sterile distilled water. The dishes were covered and the explants were

cultured at 37°C in a humidified incubator.

After the explants were cultured for one hour, 20 uCi per ml [^{14}C]-glycine (Amasham Corporation, Oakville, Ontario) with a specific activity of 52.2 mCi/mmol was added to the culture dishes. The cultures were continued for two hours after the addition of isotopes. The interparietal sutural areas (Moss, 1954) were then dissected out carefully. Each sample was placed in 1.5 ml plastic tubes and frozen after washing twice with 0.5 ml of fresh culture medium to remove free isotopes. For ages younger than two weeks, two sutures were placed in each plastic tube to constitute one sample and processed together thereafter.

Pepsin Extraction of Radio-labelled Collagen

The frozen tissues were thawed and subjected to limited pepsin digestion for collagen solubilization. Each sample was digested three times with 500 ul of pepsin (2 x crystallized, Sigma Chemical Co., St. Louis, Missouri) solution (0.1 mg/ml in 0.5 N acetic acid) at 16°C for 12 hours. After each digestion, the samples were centrifuged at 10,000 x g for 3 minutes and the supernatants were removed and frozen. The residues were resuspended in 500 ul of fresh pepsin solution for further digestion. After removel of supernatants of the third digestion, the residues were washed by resuspension in 500 ul of 0.5 N acetic acid, centrifuged and the washings removed. The supernatants and washing of each sample (total volume 2.0 ml) were pooled and mixed thor-

oughly. 100 ul of each sample was placed in mini-scintillation vials with 5 ml of scintillation cocktail (Aquasol, New England Nuclear, Boston, Massachusetts) and counted for [^{14}-C] in a liquid scintillation counter (Nuclear Chicago Isocap 300, Searle Instrumentation, Toronto, Ontario). The remaining was frozen and lyophilized in preparation for gel electrophoresis.

Collagen Separation and Quantification

Collagen and procollagen alpha-chains were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis on 20 cm slab gels. The procedure followed for the separation of those collagen components was described by Laemmli (1970) and utilized a 7.5% cross-linked separating gel, a 2.5% stacking gel and Tris/glycine buffers (Bio-Rad Laboratories, Richmond, California). Type I and type III collagen alpha-chains were separated using the interrupted electrophoresis method of Sykes and co-workers (1976).

Freeze-dried samples were dissolved in 70 ul of reservoir buffer containing 2M urea, 2% sodium dodecyl sulphate and 0.1% bromophenol blue and heated at 60°C for 30 minutes to denature the collagen. Samples were introduced to the sample wells and electrophoresis was performed for 1 hour at 130 V. Electrophoresis was stopped and samples were then reduced by the addition of 20% mercaptoethanol to the sample wells to allow type III alpha-chains to penetrate the gel. After standing for 60 minutes, the electrophoresis was continued until the tracking dye reached the base of

the gel.

For fluorographic visualization of separated radio-labelled collagen bands, gels were washed twice in dimethyl sulfoxide (Fisher Scientific, Fair Lawn, New Jersey) and impregnated with 2,5-diphenyloxazole (New England Nuclear, Boston, Massachusetts) as described by Bonner and Laskey (1974). The gels were placed on filter paper and dried on a slab drier (Bio-Rad, Richmond, California) and exposed to Kodak XRP-1 x-ray films (Kodak Canada, Inc., Toronto, Ontario) at -60°C for varying periods of time as indicated by the scintillation counts of the samples.

Individual sample tracks from the developed fluorographs were then scanned at 550 nm and proportions of type III alpha-chains relative to the total of type I and type III alpha-chains calculated on a spectrophotometer (Beckman DU-8, Toronto, Ontario). Values obtained for body weights and collagen ratios were subjected to statistical analysis using one factor factorial analysis (Steel and Jorrie, 1960).

RESULTS

The changes in the ratio of collagen type III to the total of type I and type III in mouse interparietal suture from newborn to adulthood can be represented as three phases of growth and development separated by two interphases. An initial fluctuating phase of high ratios began at newborn and continued to about 7 days old, followed by a statistically significant drop of the ratio between 7 and 20 days of life, representing the first interphase. During the second phase, from 3 to 10 weeks old, the ratio remained essentially unchanged at about 3.5%. Then, a second statistically significant drop of the ratio to almost zero occurred between 10 and 11 weeks old, representing the second interphase. Thereafter, the ratio remained at zero constituting the third phase with undetectable type III collagen.

TABLE V-1

BODY WEIGHT AND PROPORTION OF COLLAGEN TYPE III α-CHAINS TO THE TOTAL OF TYPE I AND TYPE III α-CHAINS IN MOUSE INTERPARIETAL SUTURE FROM NEWBORN TO ADULTHOOD

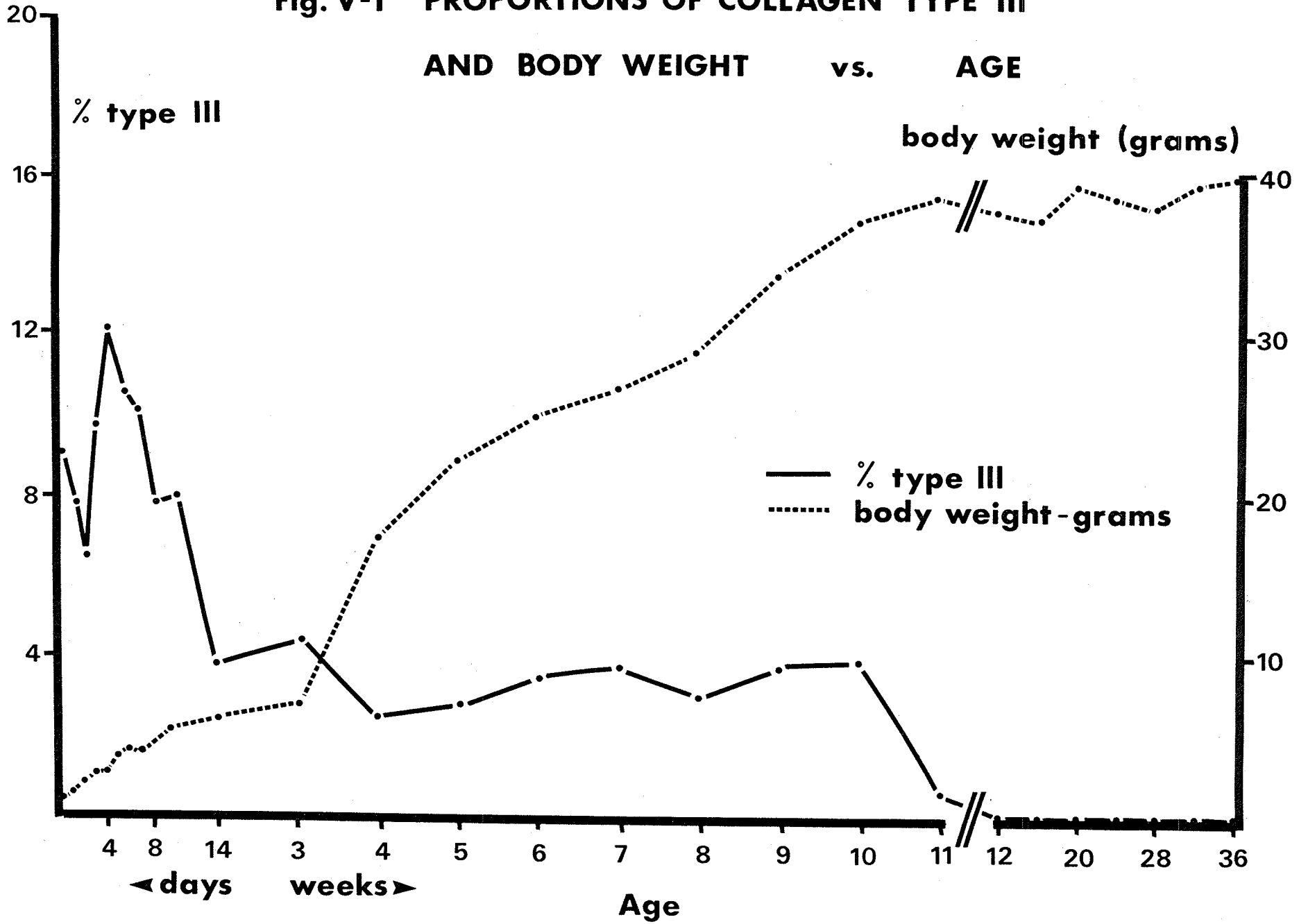
Age	N	Proportion of Type III (*) (%)	Body Weight (†) (gm)
Newborn	6	9.03	1.28
Day 1	6	8.15	1.57
2	6	6.48	1.97
3	6	9.89	2.32
4	6	12.20	2.43
5	6	10.59	3.90
6	6	10.22	4.50
7	6	7.80	4.20
10	6	8.04	5.58
Week 2	3	3.76	6.17
3	3	4.39	7.00
4	3	2.48	17.83
5	3	2.66	22.83
6	3	3.55	25.50
7	3	2.61	26.75
8	3	3.00	28.83
9	3	3.72	34.00
10	3	3.94	37.67
11	3	0.69	38.50
12	3	0.00	38.00
16	3	0.00	37.50
20	3	0.00	39.50
24	3	0.00	38.50
28	3	0.00	37.67
32	3	0.00	39.50
36	3	0.00	40.00

N = Number of mice

(*) Retransformed data. Numbers indicate means of type III ratio (%). Pooled standard error = 1.06 and applied to the means multiplicatively.

(†) Retransformed data. Numbers indicate means of body weight (gm). Pooled standard error = 1.01 from newborn to 10 days old and = 1.02 from 2 weeks of age and older and applied to the means multiplicatively.

Fig. V-1 PROPORTIONS OF COLLAGEN TYPE III AND BODY WEIGHT vs. AGE



DISCUSSION

The present findings of the growth and development of mouse interparietal suture from newborn to adulthood as indicated by the ratio of collagen type III to the total of type I and type III relates well with the histological findings of previous studies of the rat (Massler and Schour, 1951; Moss, 1954; Young, 1962; De Angelis, 1968; Ten Cate et al., 1977). A comparison with the rat is necessary because the only study of the maturation of the mouse interparietal suture to date involved mice of 3 weeks of age and older (Yen and Chiang, in press). Data of the neonatal period when growth and development is most likely the fastest are still unavailable for the mouse. As the general growth and development of rats and mice are similar (Navia, 1977) and until direct data from the mouse become available, a comparison with that of the rat seems most appropriate. Before the comparison of the biochemical and histological findings during the maturation of the interparietal suture, a review of bony tissue formation would be helpful.

When bony tissue is first formed, or when it is being rapidly laid down, for exmaple in the embryo or in the repair of wounds, the collagen in the pre-existing connective tissue and the collagen synthesized by the newly differentiated osteoblasts will together form the fibrous matrix of the new bone. The collagen fibres are of varying thickness and orientation and many are continuous with the collagen fibres of the adjacent soft connective tissue. This type of bone is termed "embryonic bone" or "coarse-fibred woven bone" and is being laid down in trabaculae or plates

which surround areas of soft connective tissue. From this starting point, embryonic bone is remodelled to form mature bone where the collagen fibres of the matrix are of even thickness and with regular orientations. However, mature bone can also be formed by direct appositional growth upon pre-existing bony surfaces without going through the "coarse-fibred woven bone" stage.

Using vital staining of bone with Alizarine red "S", Massler and Schour (1951) demonstrated that in the rat, the rate and the length of the period of rapid growth of the different cranial sutures were different. Moreover, within a suture, the rate of growth changes tremendously during this period of rapid growth. With reference to the interparietal suture, Table V-3 can be constructed from Massler and Schour's (1951) data. Figure V-2 was obtained by superimposing the growth rate (mm/day) histogram upon the collagen type III ratio with reference to age. The close relationship of the two becomes apparent indicating the ratio of collagen type III has an intimate relationship with the rate of sutural bony growth.

TABLE V-3

POST-NATAL INTERPARIETAL SUTURAL BONY GROWTH AT DIFFERENT AGES IN THE RAT

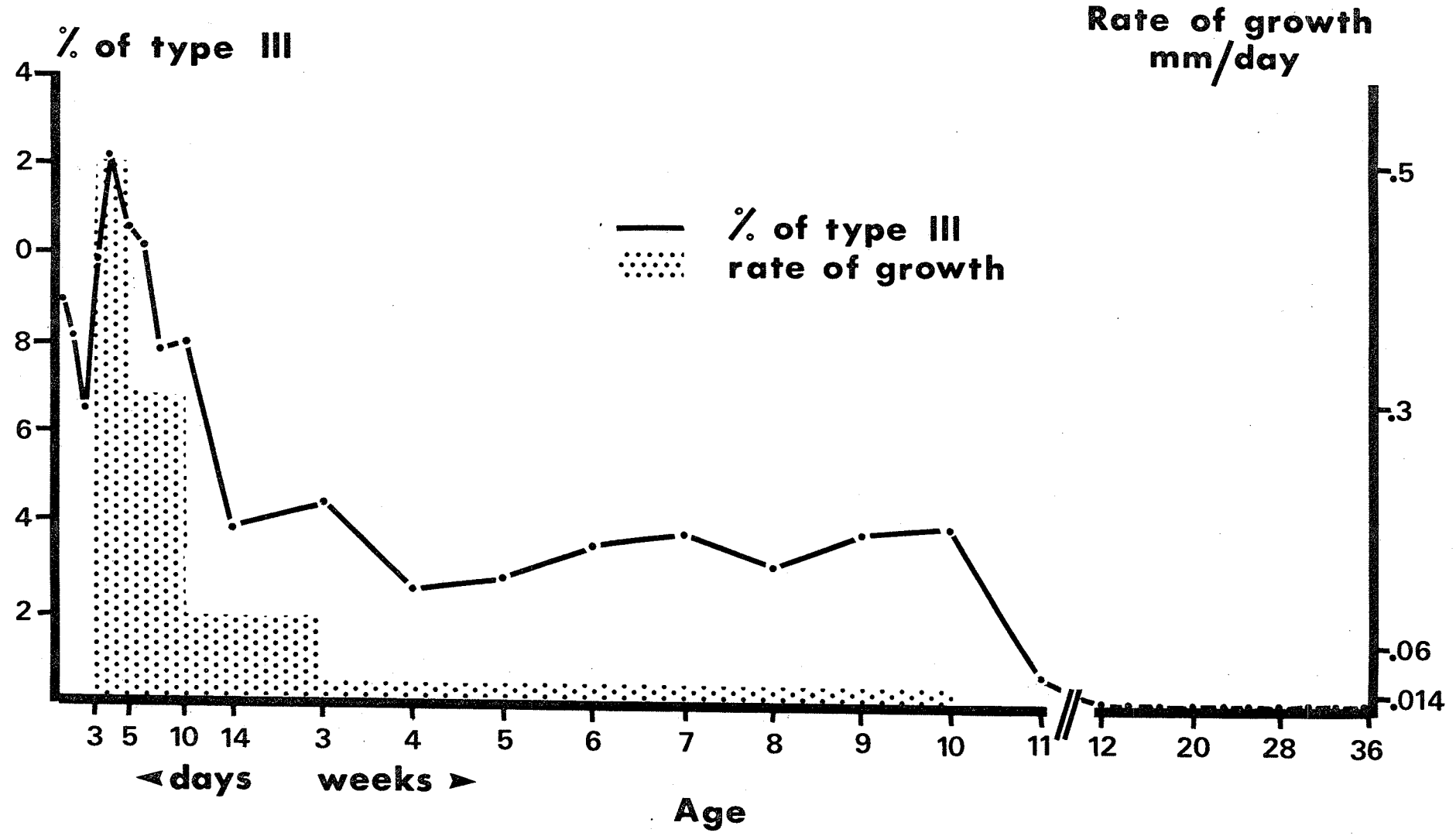
Period (Days of Age)	Interval (Days)	Amount of Growth (mm)	Rate of Growth (mm/Day)	Rate of Growth (% of Total Growth/Day)	Cumulative Amount of Growth (mm)	Cumulative % of Total Post-Natal Growth
0-3	3	*	0.00	0.00%	0.0	0.00%
3-5	2	1.0	0.50	13.51%	1.0	27.03%
5-10	5	1.5	0.30	8.11%	2.5	67.57%
10-15	5	0.3	0.06	1.62%	2.8	75.68%
15-20	5	0.3	0.06	1.62%	3.1	83.78%
20-40	20	0.2	0.014	0.38%	3.3	89.19%
40-70	30	0.5			3.8	102.70%
70-100	30	-0.1	0.00	0.00%	3.7	100.00%

* Amount of growth too small to be measured grossly.

Table constructed with data from Massler and Schour (1951)

Fig. V-2 PROPORTION OF COLLAGEN TYPE III vs. AGE IN MOUSE

RATE OF INTERPARIETAL SUTURAL GROWTH vs. AGE IN MOUSE



Young (1962) reported a post-natal period of rapid growth in the rat interparietal suture that ends around the 6th to 7th day of life. During this period, growth of the immature parietal bones takes place by the progressive, rapid addition of new matrix at the sutural edges of the bones. The margins advance by extension of trabecular woven bone, indicated by the periodic acid-Schiff staining (Curran and Collins, 1957), into the proliferating sutural tissue. De Angelis (1968) made similar findings and reported generalized intramembranous growth of embryonic bone dominates the growth process of the cranium from the second to the 8th day of life. This also coincides with Moss's (1954) description of the rapid growth period which ends around the 7th day of life for the rat interparietal suture. This extreme rapid growth during the first week of life accounts for half of the total post-natal sutural bony growth of the rat interparietal suture (Massler and Schour, 1951). Also, this is the period of sutural histodifferentiation and sutural bony edge interaction which appears to be essential for the determination of the locations of cranial sutures and a change from the embryonic to the appositional type of bony growth (Moss, 1954). Growth of the neurocranium up to 7 days was shown to be a result of generalized intramembranous embryonic bone formation centrifugally from the centres of ossification towards the presumptive sutural areas (Moss, 1954; De Angelis, 1968). Also, a given sutural line begins as the smooth periphery of a bone. As growth continues at the free margins of the bone and eventually reaches the threshold proximity with the adjacent bony edge, these margins become serrated and interdigi-

tated, indicating interaction, histodifferentiation or transformation of cellular elements must have happened, though the cause responsible for these changes is still unclear. However, extirpation experiments by Moss (1954) indicated that the location of the cranial sutures only becomes definitive after this bony edge interaction has taken place and was found to be at the 7th day of life for the rat interparietal suture. During this period, the sutural soft tissue also undergoes certain definitive transformation (Moss, 1954). The sutural soft tissue becomes increasingly cellular and packing and crowding of these cells are evident as its extent is diminished with the approaching of the reciprocally bevelled bony edges.

Thus, there is a close relationship between the high collagen type III ratio with rapid embryonic bone formation, sutural histodifferentiation and sutural bony edge interaction, although the nature of this relationship as well as suggestions of a cause and effect nature require further studies.

During the second and third week of life, the rate of bony growth was shown to decrease rapidly (Massler and Schour, 1951) and the trabecular mode of woven bone osteogenesis gave way to slower lamellar appositional growth at the sutural edges (Moss, 1954; Young, 1962). But this period of two weeks still accounts for about a third of the total post-natal bony growth of the rat interparietal suture (Massler and Schour, 1951), though a relatively greater emphasis is given to increasing bone thickness and to internal reorganization (Moss, 1954; Young, 1962; De Angelis, 1968). Also, there is a differential pattern of apposition at the

bony edge of the suture, resulting in a progressive relocation of the bony edge in an ectocranial direction as it expands into the suture (Young, 1962; De Angelis, 1968). The non-osseous area of the suture is now much narrower and by the end of the third week, assumes the three-layered structure described by Weinmann and Sicher (1947) (Moss, 1954).

Thus, this is a period characterized by changes, rate of growth, mode of growth, bony reorganization and suture structural changes. All these correspond with a transition from high to low collagen type III ratios (Figure V-2). Again, what factors and control mechanisms are responsible for the biochemical and histological changes during this period are still unknown.

The next stage of growth and development of the interparietal suture is characterized by a state of uniformity, both in terms of collagen type III ratios and histological changes of the suture. The collagen type III ratio remains at around 3.5% from 3 weeks to 10 weeks of age and is associated with the slow appositional growth of the remaining 15% of post-natal sutural bony growth (Massler and Schour, 1951). Also, the general increase in bone thickness and the ectocranial migration of the suture continue with the resultant flattening of the calvarium (Young, 1962; De Angelis, 1968).

After the 70th day of life, growth at the suture ceases completely (Massler and Schour, 1951; Moss, 1954) accompanied by the progressively fibrous nature of the suture and the precipitous drop of collagen type III ratio to zero. Thus, a stable, but active, interparietal suture in Swiss male white mouse, suitable

for the study of collagen remodelling would be between 9 and 10 weeks old as growth is practically complete, but before the fibrous transformation and disappearance of collagen type III have taken place.

The lack of rapid growth during the first three days of life, which is common to all cranial sutures in the rat (Massler and Schour, 1951) and coincides with a statistically significant drop in the collagen type III ratio in the mouse interparietal suture, is puzzling and without explanation at present. The sudden change from in utero to animal room environment may have some contribution, but is unlikely to be solely responsible.

The changes in the collagen phenotype ratio does not seem to relate to changes in body weight during growth and development (Figure V-1) suggesting the interparietal suture may have a maturing pattern of its own. Considering the close relationship between the cranium and the neural tissues it encompasses and protects, the growth pattern of cranial sutures would likely be more in tune with that of neural tissues rather than the general skeletal growth (Young, 1959).

There are great variations in the rate and the length of the period of rapid growth among the different cranial sutures in rats (Massler and Schour, 1951). The frontonasal suture is of particular interest in this respect. It has both the fastest rate of growth and the longest period of growth extending up to and possibly beyond the 300th day of life (Massler and Schour, 1951; Moss, 1954). Also, the timetable for cellular histodifferentiation and interaction seem to be different as the histological changes in

the sutural structure during the third week of life in the interparietal suture do not occur in the frontonasal suture until well past the third month (Moss, 1954). A similar comparison of the biochemical and histological events in the frontonasal suture during growth and development may add insight into the relationship between type III ratio and other metabolic phenomenon.

Finally, descriptive and correlation accounts of events happening during growth and development do not explain the regulation and control mechanisms responsible for the histodifferentiation and morphogenesis. However, the identification of a metabolic parameter sensitive to growth and developmental changes is a step closer to this explanation and understanding.

CONCLUSIONS

1). The ratio of collagen type III to the total of type I and type III has a specific but non-linear relationship with age.

2). The ratio at birth is high, followed by a statistically significant drop during the first two days of life. This corresponds with the lack of sutural growth generally during this immediate post-natal period.

3). Day 3 to day 7 represents a period of very high type III ratios, reaching a plateau of about 12% and is the period during which the rat interparietal suture exhibits rapid embryonic bone formation, sutural histodifferentiation and sutural bony edge interaction. This period of about four days represents about half of the total post-natal interparietal sutural bony growth in the rat.

4). A first interphase, from 7th to 21st days of life, represents a period of transition from high to low type III ratio. This is a period of change of the metabolic state of the suture: mode of growth, rate of growth, bony reorganization and suture structural changes. This period of about fourteen days represents about one-third of the total post-natal interparietal sutural bony growth in the rat.

5). A phase of steady metabolic activity from 3 weeks to 10

weeks of age with a fairly constant type III ratio of around 3.5%. This period of 7 weeks represents the remaining 15% of post-natal sutural bony growth and the progressive thickening and flattening of the calvarium.

6). A second interphase with a precipitous drop of the type III ratio to almost zero occurs during the 11th week of life representing suture maturation.

7). The ratio of collagen type III has an intimate relationship with the metabolic state and the rate of sutural bony growth.

8). The changes in the collagen phenotype ratio does not relate to changes in body weight during growth and development, suggesting the interparietal suture may have a maturing pattern of its own.

9). The desirable age for orthopaedic studies in Swiss male white mouse interparietal suture would be between 9 and 10 weeks of age.

CHAPTER VI

TIME-COURSE STUDY OF COLLAGEN PHENOTYPE SYNTHESIS IN MOUSE INTERPARIETAL SUTURE UNDER TENSILE STRESS OF VARYING FORCE LEVELS *IN VITRO*

SUMMARY

Nine week old Swiss male white mice were divided into groups sacrificed after time intervals of force application of 6 hours and 1, 3, 5, 7, 10, 14, 21 and 28 days. Each group had 21 animals: 3 age control, 3 sham-operated and 3 experimental animals for each of the five force levels: 50 gm, 35 gm, 25 gm, 15 gm and 5 gm. The experimental animals had helical springs placed surgically in their calvaria to expand the interparietal suture. The sham-operated animals received the same surgical procedure, but without placement of springs. The age controls were animals with the same age as the experimental and sham-operated animals at the time of sacrifice.

After sacrifice, the amount of sutural expansion was measured and calvaria with the implanted springs were dissected from the mice and the explants cultured in Trowell-type organ culture dishes. [^{14}C]-glycine was added sixty minutes after the beginning of *in vitro* culture and all explant cultures were then continued for two hours after the addition of isotopes. The calvaria from the sham-operated and age control animals were cultured similarly. The interparietal sutural tissues were then dissected for collagen extraction by limited pepsin digestion. Using SDS-polyacrylamide gel electrophoresis and fluorographic visualization of separated radio-labelled collagen bands, the time course collagen type III ratios in the mouse interparietal suture under tensile stress of the five force levels were determined. All the experimental and sham-operated animals responded with a rapid rise followed by an

almost equally rapid fall in the type III ratio before stabilized at a level that is significantly higher than that of the age controls for the rest of the experimental period. The increase in the type III ratio at 6 hours were significantly higher for lighter forces than for heavier forces. The peak value of the ratio due to the lightest force was more compatible with the ratios that occur naturally in the suture during rapid growth and development than those due to the heavier forces. Thus, it appeared that light forces tend to initiate a more physiologic response than heavy forces with respect to the collagen type III ratio.

The rate of suture expansion was directly proportional to the force value of the tensile stress. However, a 2.0 mm expansion was achieved for all at the end of four weeks.

INTRODUCTION

With the exception of the mandible, the clinical practice of orthodontics and facial orthopaedics is the controlled mechanical manipulation of fibrous joints (sutural and periodontal) of the craniofacial complex via the process of biologic adaptation, termed remodelling. Much effort has been expended in the past with the objective of understanding the relationship between the application of mechanical stress and the resultant histological and morphological changes in fibrous joint remodelling. The ultimate clinical aim is the efficient achievement of therapeutic objectives with the minimal treatment sequalae or iatrogenic damage.

A pathway of cellular events required for periodontal remodelling has been suggested by Melcher (1980) in which the initial stimulus (in this case mechanical stress) is first transduced to a biological signal or signals whose targets are the progenitor cells. These cells will eventually differentiate into those specialized cells capable of remodelling the periodontium. However, the process of transduction, differentiation, migration and control of activity of the progenitor cells are still largely unknown. Also, while extrinsic mechanical manipulation is the only treatment modality available at present, no biologically based rationale has been proposed for selection of force magnitude, and parameters representing optimal use of mechanical stress for orthodontic and facial orthopaedic purposes are still to be explored. This is why orthodontics and facial orthopaedics as

practised today are still rather intuitively and empirically based: more of an art than a science. Thus, even though remodelling is part of the normal biologic activity of fibrous joints throughout life, orthodontically or orthopaedically induced remodelling is often accompanied by a variable amount of periodontal destruction, usually in the form of root resorption (Harry and Sims, 1982), iatrogenic sequalum of present day orthodontics (Zaccharison, 1976). While the nature of equilibrium or lack of equilibrium of the force system acting upon the dentition and the dentoalveolar process is still unknown; a disturbance of this force system, however, often leads to the various forms of malocclusion. The development of anterior openbite, extreme proclination of upper anteriors or constricted V-shaped maxillary dental arch form as a result of anterior tongue thrust, thumb-sucking or mouth breathing habit respectively, necessitates a tremendous amount of remodelling of the alveolar bone and the periodontal ligament. Yet, periodontal destruction in the form of root resorption is extremely rare in these instances. This may suggest that orthodontic tooth movement without accompanying treatment sequalae is theoretically possible if our extrinsic force systems operate within the limits of the intrinsic force systems that resulted in the development of malocclusions. Thus, study of the differences in the response of fibrous joint remodelling with respect to different levels of mechanical stress could be of importance.

Collagen is the main constituent of fibrous joints and its synthesis and secretion by fibroblasts is a tightly controlled

process under constant influence of extracellular conditions (Hance and Crystal, 1977; Muller et al., 1981; Saltzmen et al., 1982) including the immediate mechanical environment (Leung et al., 1976, 1977; Yen et al., 1980; Meikle et al., 1982). There is much evidence suggesting that mechanical perturbation of the plasma membrane of connective tissue cells initiates specific biochemical changes within the cell (Rodan et al., 1975; Bourret and Rodan, 1976; Hang et al., 1976; Norton et al., 1977; Somjen et al., 1980). Also, modulated synthesis of the several collagens may be crucial for the normal progression of dynamic processes such as development and tissue repair (Bornstein and Sage, 1980). Changes in the collagen phenotype ratio, especially that of type I and type III, may serve as an important parameter indicating the metabolic state of the connective tissue involved, and may act as a signal for the change in cellular activities (Chung and Miller, 1974; Epstein, 1974; Butler et al., 1975; Weiss et al., 1975; Miller, 1976; Reddi et al., 1977; Gay et al., 1978). The changing ratio of collagen type III to the total of type I and type III has a specific relationship with the stage of growth and development in the mouse interparietal suture (see Chapter V). Also, a change in collagen phenotype synthesis, with an increase in the type III ratio as a result of mechanical stress was demonstrated in the interparietal suture of adolescent mouse (Yen et al., 1980; Chapter III) and neonatal rabbit (Meikle et al., 1982).

The purpose of the present investigation is to determine the changes in the type III ratio of the newly synthesized collagens with time, in adolescent mouse interparietal sutures when sub-

jected to tensile stress of different force levels and to compare these time-course type III ratio profiles with that of the suture during rapid growth and development.

MATERIALS AND METHODS

Expansion Springs and Force Levels

Expansion springs of five different force levels were formed on templates with the following material:

a). 50 gm springs with 0.016" Tru-chrome orthodontic wire (Rocky Mountain/Orthodontics, Denver, Colorado).

b). 35 gm springs with 0.014" Tru-chrome orthodontic wire (Rocky Mountain/Orthodontics, Denver, Colorado).

c). 25 gm springs with 0.014" TMA orthodontic wire (Ormco, Glendora, California).

d). 15 gm springs with 0.012" Tru-chrome orthodontic wire (Rocky Mountain/Orthodontics, Denver, Colorado).

e). 5 gm springs with 0.009" Red Elgiloy orthodontic wire (Rocky Mountain, Denver, Colorado).

All springs were about the same size (6 mm x 7 mm) and delivered the designated tensile stress $\pm$ 5% with a 2.0 mm activation.

Surgical Technique

Nine week old Swiss male white mice, inbred in our facility, were divided into groups sacrificed after time intervals of force application of 12 hours, and 1, 3, 5, 7, 10, 14, 21, 28 days. Each group had 21 animals: 3 control, 3 sham-operated and 3 experimental animals for each of the five force levels.

Surgery was performed after mice were anaesthetized with a dosage of 0.07 mg/gm body weight of sodium pentobarbital (Nembutal, Abbott Laboratories, Montreal, Quebec), plus a local injection of xylocaine (2% xylocaine, 1:50,000 epinephrine, Astra Laboratories, Mississauga, Ontario) underneath the scalp. A mid-sagittal incision was made through the scalp exposing the calvaria. Holes were drilled through the calvaria 2.0 mm on either side of the interparietal suture, using a 1/4 round burr (Jet carbide burrs, Beavers Dental Products Ltd., Morrisburg, Ontario) mounted in a highspeed handpiece. Expansion springs with a 2.0 mm activation were implanted by inserting the spring legs through the holes in the calvaria. The skin flaps were reapposed and the incision closed with 4.0 black silk suture (Ethicon, Peterborough, Ontario). Sham-operated animals had surgery performed similarly, but without the implantation of springs. Animals of the same age as the sham-operated and experimental animals at the time of sacrifice served as controls.

Dissection and In Vitro Calvaria Organ Explant Culture System

After the respective time periods of force application, the mice were killed by cervical dislocation. The amount of sutural expansion was measured with a millimeter ruler and calvaria with the implanted springs were dissected from the mice and any opposing soft tissues were carefully removed. Using sharp dissecting scissors, each calvaria was trimmed to a size of about 10 x 12 mm

with the interparietal suture in the longitudinal midline of the explant.

The explants were placed on stainless steel grids (Falcon #3014, Becton, Dickinson and Co., Oxnard, California) at the gas (mixture of 95% O2 and 5% CO2 humidified by bubbling thorugh distilled water) to medium (Waymouth's 752/1, Grand Island Biological Co., Grand Island, New York) interphase in Trowell-type (Trowell, 1959) organ culture dishes (60 x 15 mm, Falcon #3037, Becton, Dickinson and Co., Cockeysville, Maryland) containing 800 ul of supportive medium (Waymouth, 1959) supplemented with 300 ugm/ml ascorbic acid, 330 U/ml penicillin, 0.75 ugm/ml amphotericin B and 330 ugm/ml streptomycin. The centre wells of the dishes were surrounded by absorbent filter-paper rings moistened with sterile distilled water. The dishes were covered and the explants were cultured at 37°C in a humidified incubator.

After the explants were cultured for one hour, 20 uCi per ml. [^{14}C]-glycine (Amasham Corporation, Oakville, Ontario), with a specific activity of 52.2 mCi/mmol, was added to the culture dishes. In vitro culture was continued for two hours after the addition of isotopes. The interparietal sutural areas (Moss, 1954) were then dissected out carefully. Each sample was placed in 1.5 ml plastic tubes and frozen after washing twice with 0.5 ml of fresh culture medium to remove free isotopes.

Pepsin Extraction of Radio-labelled Collagen

The frozen tissues were thawed and subjected to limited pepsin digestion for collagen solubilization. Each sample was digested three times with 500 ul of pepsin (2 x crystallized, Sigma Chemical Co., St. Louis, Missouri) solution (0.1 mg/ml in 0.5 N acetic acid) at 16°C for 12 hours. After each digestion, the samples were centrifuged at 10,000 x g for 3 minutes and the supernatants were removed and frozen. The residues were resuspended in 500 ul of fresh pepsin solution for further digestion. After removal of supernatants of the third digestion, the residues were washed by resuspension in 500 ul of 0.5 N acetic acid, centrifuged and the washings removed. The supernatants and washing of each sample (total volume 2.0 ml) were pooled and mixed thoroughly. 100 ul of each sample was placed in mini scintillation vials with 5 ml of scintillation cocktail (Aquasol, New England Nuclear, Boston, Massachusetts) and counted for [^{14}-C] in a liquid scintillation counter (Nuclear Chicago Isocap 300, Searle Instrumentation, Toronto, Ontario). The remaining was frozen and lyophilized in preparation for gel electrophoresis.

Collagen Separation and Quantification

Collagen and procollagen alpha-chains were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis on 20 cm slab gels. The procedure followed for the separation of those

collagen components was described by Laemmli (1970) and utilized a 7.5% cross-linked separating gel, a 2.5% stacking gel and Tris/glycine buffers (Bio-Rad Laboratories, Richmond, California). Type I and type III collagen alpha-chains were separated using the interrupted electrophoresis method of Sykes and co-workers (1976). Freeze-dried samples were dissolved in 70 ul of reservoir buffer containing 2 M urea, 2% sodium dodecyl sulphate and 0.1% bromophenol blue and heated at 60°C for 30 minutes to denature the collagen. Samples were introduced to the sample wells and electrophoresis was performed for 1 hour at 130 V. Electrophoresis was stopped and samples were then reduced by the addition of 20% mercaptoethanol to the sample wells to allow type III alpha-chains to penetrate the gel. After standing for 60 minutes, the electrophoresis was continued until the tracking dye reached the base of the gel.

For fluorographic visualization of separated radio-labelled collagen bands, gels were washed twice in dimethyl sulfoxide (Fisher Scientific, Fair Lawn, New Jersey) and impregnated with 2,5-diphenyloxazole (New England Nuclear, Boston, Massachusetts) as described by Bonner and Laskey (1974). The gels were placed on filter paper and dried on a slab drier (Bio-Rad, Richmond, California) and exposed to Kodak XRP-1 x-ray films (Kodak Canada, Inc., Toronto, Ontario) at -60°C for varying periods of time as indicated by the scintillation counts of the samples.

Individual sample tracks from the developed fluorographs were then scanned at 550 nm and proportions of type III alpha-chains, relative to the total of type I and type III alpha-chains, were

TABLE V

ANALYSIS OF VARIANCE OF MEAN VALUES OF RAPE III/TYPE I + III α-CHAINS IN THE MOUSE INTERPARIETAL SUTURE DURIST FOUR WEEKS OF LIFE (†)

	Newborn = 9.03	Day 1 = 8.15	Day 2 = 6.48	Day 3 = 9.89	Day 4 = 12.20	Day 6 = 10.22	Day 7 = 7.80	Day 10 = 8.04	Week 2 = 3.76	Week 3 = 4.39	Week 4 = 2.48
Newborn = 9.03		NS	**	NS	**	NS	NS	NS	**	**	**
Day 1 = 8.15	NS		**	**	**	**	NS	NS	**	**	**
Day 2 = 6.48	**	**		**	**	**	NS	NS	**	**	**
Day 3 = 9.89	NS	**	**		**	NS	**	**	**	**	**
Day 4 = 12.20	**	**	**	**		**	**	**	**	**	**
Day 5 = 10.59	*	**	**	NS	**	NS	**	**	**	**	**
Day 6 = 10.22	NS	**	**	NS	**		**	**	**	**	**
Day 7 = 7.80	NS	NS	NS	**	**	**		NS	**	**	**
Day 10 = 8.04	NS	NS	NS	**	**	**	NS		**	**	**
Week 2 = 3.76	**	**	**	**	**	**	**	**		NS	NS
Week 3 = 4.39	**	**	**	**	**	**	**	**	NS		*
Week 4 = 2.48	**	**	**	**	**	*	**	**	NS	*	

(†) Retransformed data. Numbers indicate means of type + III (%).
Pooled standard error = 1.06 and applied to the mearatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

calculated on a spectrophotometer (Beckman DU-8, Toronto, Ontario). Values obtained for sutural expansion and collagen ratio were subjected to statistical analysis using two factor factorial analysis (Steel and Jorrie, 1960) and multiple t-test analysis.

OBSERVATIONS AND RESULTS

Both sham-operated and experimental animals recovered quickly from the effect of anaesthesia and the operating procedure and started drinking and feeding normally in about 2-4 hours. The implanted springs were well tolerated by the mice with no instance of post-surgical infection.

Tensile stress applied across the interparietal suture induced a rapid lateral movement of the interparietal bones with obvious widening of the suture. The normal structure of the suture was changed fromm a thin, dark band to a translucent sheet of connective tissue, still connecting the parietal bones. The rate of separation was directly related to the magnitude of the tensile stress (Table VI-1 and Figure VI-1). The average amount of sutural expansion due to tensile stress of the five different force levels at each of the nine designated sacrifice time intervals were compared with multiple t-test analysis (Tables VI-2 to VI-10 in Appendix) . It was also observed that the anterior lambdoidal sutures, coronal sutures and metopic sutures of all experimental animals were stretched at varying degrees.

At the end of the experimental period (4 weeks), calvarial morphology was significantly changed, both in form and size. The calvaria were obviously enlarged with general thickening of the parietal bones, especially at the lateral aspects of drilled holes.

The average ratios of type III alpha-chains relative to the total of type I and type III alpha-chains under the various exper-

imental conditions were summarized in Table VI-11 and Figure VI-2. Analysis of variance indicated a significant difference in the mean values of ratios of type III collagen over the experimental period for both the sham-operated and all of the tensile stressed sutures (Table VI-12 to VI-18 in Appendix). Also, multiple t-test analysis of the mean values of ratios of type III collagen at each of the nine designated sacrifice time intervals indicated a statistically significant relationship between the type III ratio and the force level of the tensile stress (Table VI-19 to VI-27 in Appendix).

TABLE VI-1

SUTURE EXPANSION (MM) AFTER INTERVALS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

D. After Spring Force Implant Levels	6 hours	1 day	3 days	5 days	7 days	10 days	14 days	21 days	28 days
50 gm	0.67	1.00	1.22	1.56	1.67	2.00	2.00	2.00	2.00
35 gm	0.50	0.67	1.33	1.33	1.50	1.56	1.84	2.00	2.00
25 gm	0.50	0.67	1.00	1.11	1.33	1.50	1.78	1.89	2.00
15 gm	0.33	0.33	0.67	1.00	1.33	1.33	1.61	1.89	2.00
5 gm	0.00	0.00	0.44	0.56	0.50	0.78	1.33	1.50	2.00

(†) Retransformed data. Numbers indicate means of expansion (mm). Pooled standard error = 1.01 and applied to the means multiplicatively.

NOTICE/AVIS

PAGE(S) 132 - 134 ~~IS~~/ARE ~~EST~~/SONT colour photos

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Fig. VI-1 SUTURE EXPANSION (mm) AFTER INTERVALS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS

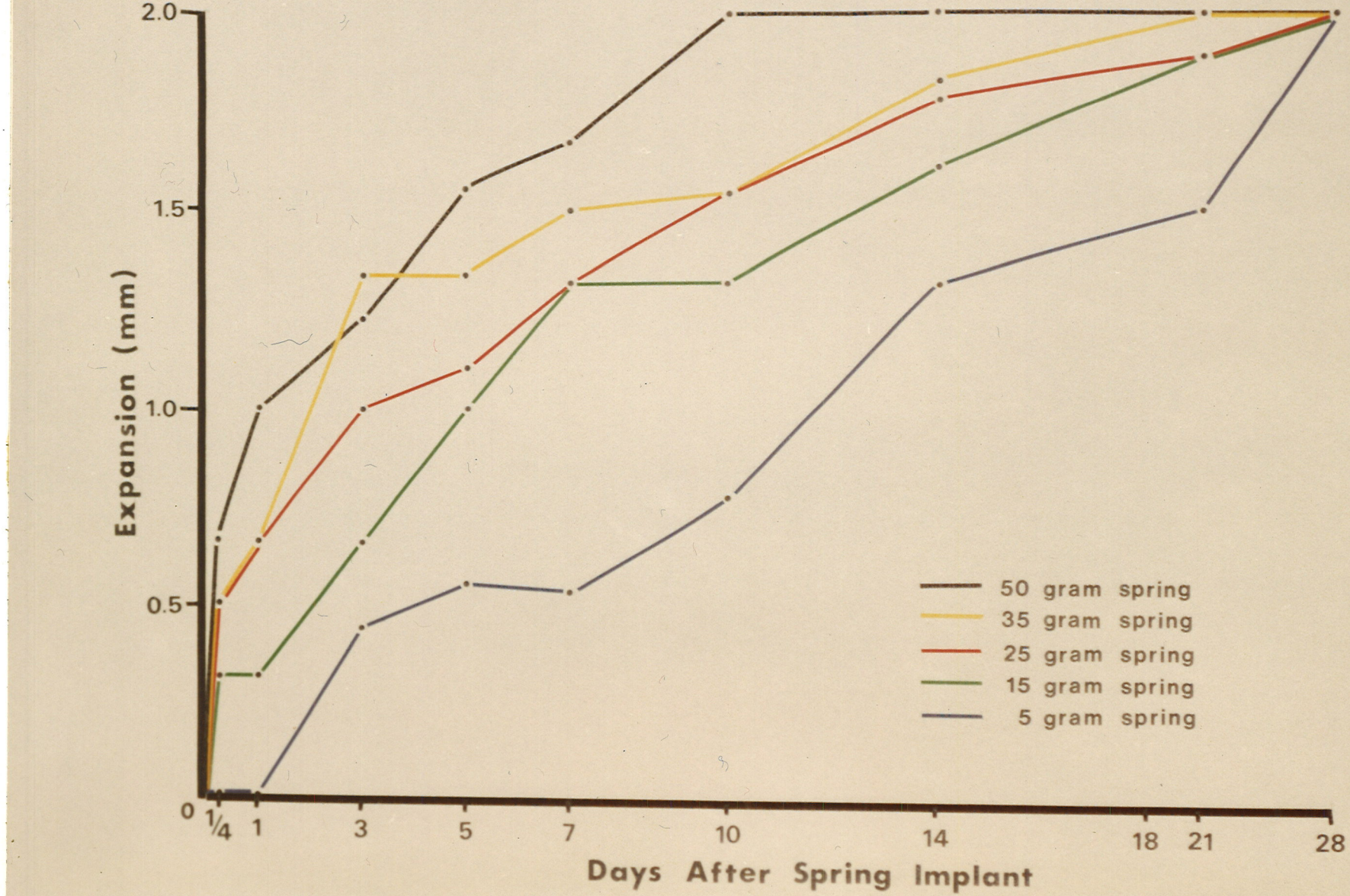


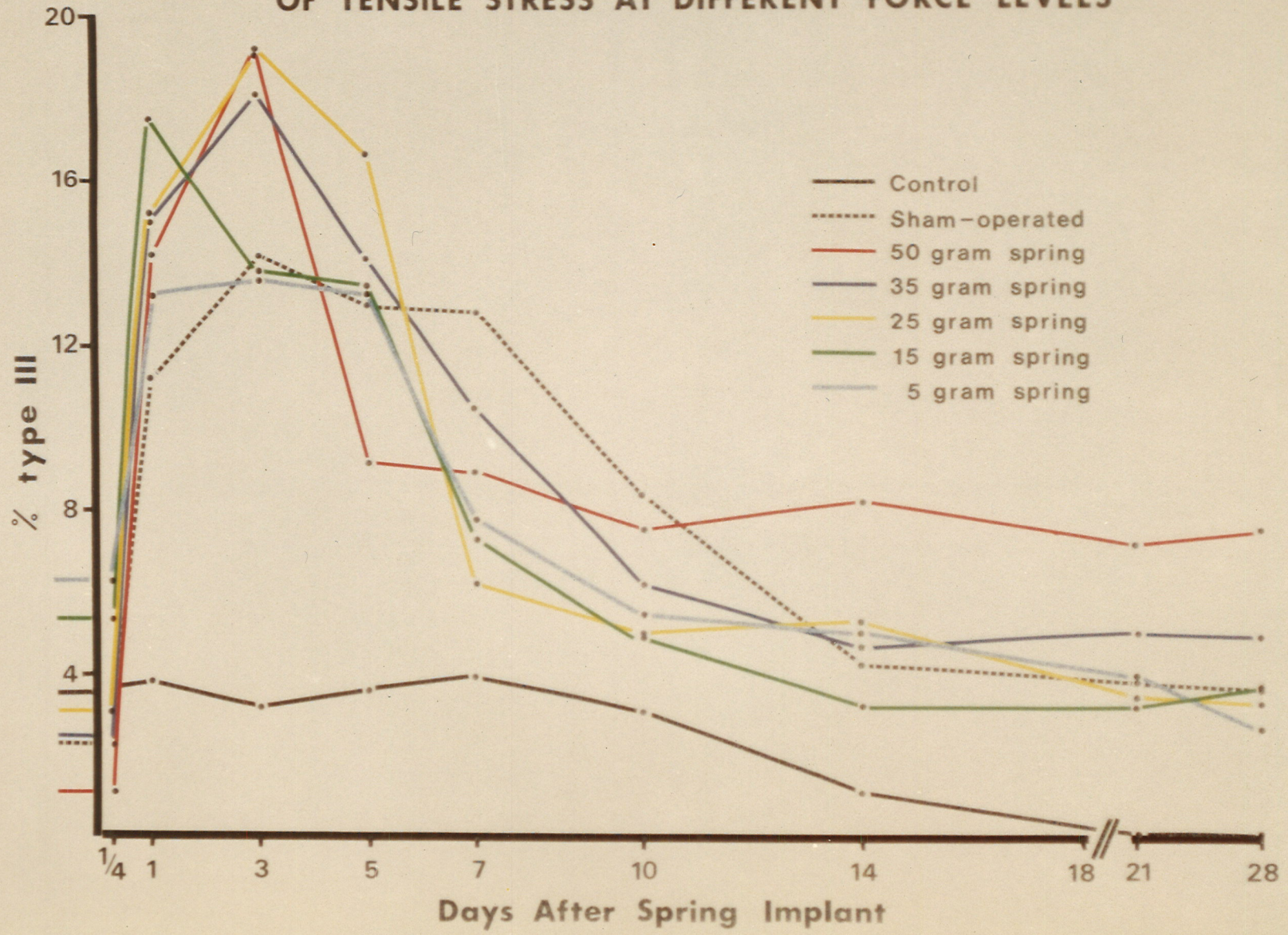
TABLE VI-11

PROPORTIONS OF TYPE III COLLAGEN AFTER INTERVALS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

D. After Spring Implant / Treatment	6 hours	1 day	3 days	5 days	7 days	10 days	14 days	21 days	28 days
Control	3.72	3.82	3.27	3.69	3.94	3.00	0.69	0.00	0.00
Sham-operated	2.29	11.29	14.10	12.92	12.53	8.39	4.24	3.82	3.61
50 gm	1.08	14.82	19.38	9.05	8.77	7.62	8.10	7.19	7.58
35 gm	2.49	14.96	18.05	14.07	10.53	6.10	4.57	4.93	4.72
25 gm	3.08	15.06	19.33	16.38	6.21	4.98	5.30	3.55	3.28
15 gm	5.42	16.85	13.85	13.34	7.23	4.97	3.21	3.26	3.65
5 gm	6.11	13.21	13.64	13.24	7.79	5.45	5.14	3.84	2.39

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

Fig. VI-2 PROPORTIONS OF TYPE III COLLAGEN AFTER INTERVALS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS



DISCUSSION

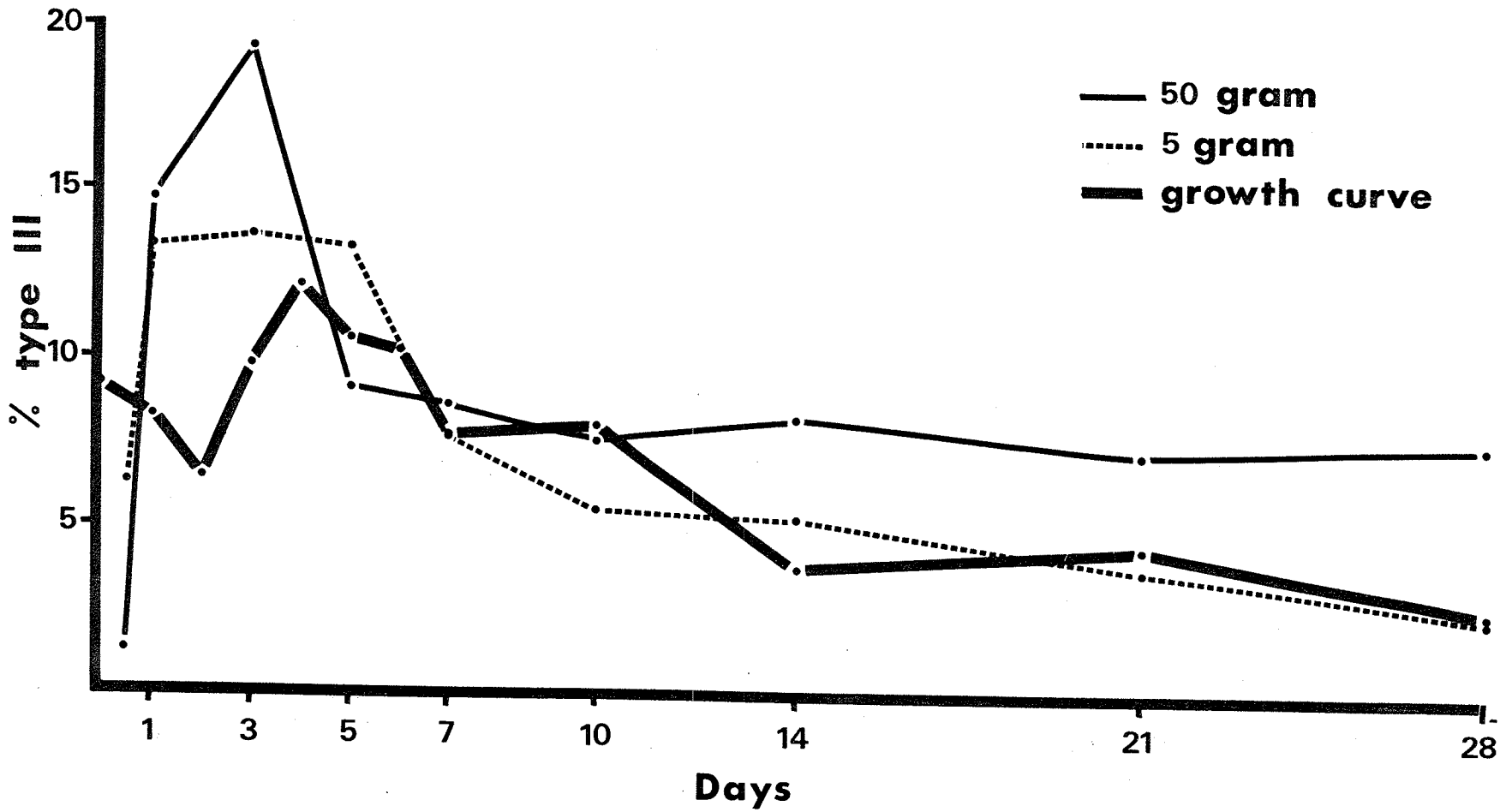
It has often been mentioned that teeth are responsive to orthodontic forces, while orthopaedic forces are required for craniofacial manipulation. This tends to suggest that there are inherent differences between the periodontal and sutural joints with respect to their response to extrinsic mechanical stress. However, this is in contradiction to the fact that both are fibrous joints and that they share many common features, both in structure and in function. The magnitude of the extrinsic force applied may not be the best representation of the mechanical stress experienced at the cellular level. A better representation would be in terms of force/unit area of tissue under stress. The obvious problem here is technology.

The springs used in the present experiment were accurately calibrated to within 5% of the stated force levels. However, the mechanical stress per unit area of the interparietal suture was unknown. It was difficult to determine the cross-sectional area of the suture. Also, portions of the stress were transmitted and supported by other sutures of the calvaria (Isaacson, 1964; Zimring and Isaacson, 1965; Moffett, 1971, 1973; Nanda, 1978). In addition, the force levels decreased rapidly as the sutures were expanded because of the high load deflection ratio of the springs, as the dimensions of the springs were restricted by the size of the mouse calvaria. Nevertheless, it is reasonable to assume that a decreasing level of tensile stress was delivered to the mouse interparietal suture from the heavier to the lighter helical

springs. Passive springs were not inserted into sham-operated controls due to the inability to secure them to the calvaria.

The collagen type III ratio profiles over the time period studied demonstrated similarities and differences in the response of the mouse interparietal suture with respect to the treatment received and the force levels of the tensile stress. All the experimental and sham-operated animals responded with a rapid increase of type III ratio, reaching a maximum at 3 days (except the maximum ratio for the 15 gm force was at 1 day). There was almost an equally rapid decrease of the ratio between day 3 and day 7, followed by a more gradual decrease during the following week. The ratios then stabilized at a level significantly higher than that of the age controls for the rest of the experimental period. This general pattern parallels findings in situations whenever rapid extracellular matrix production is necessary, as in embryonic tissues (Epstein, 1974); wound healing (Barnes et al., 1976; Gay et al., 1978) and matrix-induced bone formation (Reddi et al., 1977; Steinmann and Reddi, 1980). Moreover, they are remarkably similar to the type III ratio profile in the suture during the first four weeks of life when rapid growth and development is taking place (Figure VI-3). However, the differences in response with respect to the different force levels of the tensile stress are of more importance here.

Fig. VI-3 TYPE III RATIO vs. TIME IN DAYS



The increases in the type III ratio at 6 hours were significantly higher for lighter forces than for heavier forces, though no actual suture expansion can be measured for the 5 gm force. This seems to suggest that tensile stress, without causing excessive tissue damage, is best able to initiate changes in collagen type III ratio. If a high type III ratio implies histodifferentiation and rapid growth as suggested by the findings in the growth study of the suture (Chapter V), then lighter forces can better initiate this remodelling process. The ratios of type III at 6 hours for the heavier forces were actually lower than that of the age controls and may partly be due to the trauma experienced by the sutural tissue with the application of heavy forces as an expansion of more than 0.5 mm was noted at 6 hours with 50 gm springs. Tissue damage with haemorrhage from blood vessels may delay the synthesis of type III collagen as platelet derived growth factor present in plasma has an inhibitory effect on collagen type III synthesis (Narayanan and Page, 1983b).

The maximum value of the type III ratio depends on the force level of the tensile stress. The maximum ratios due to heavier forces are much higher than that of the lightest force (Figure VI-2). However, the high ratios that occur naturally in the suture during rapid growth and development are much closer to the maximum ratio due to the lightest force used in the present investigation than those due to the heavier forces (Chapter V, Figure VI-3). In addition, during the period when the ratios were stabilized (day 14 to day 28), the ratios of the lighter forces are very close to that of the period during growth and development (3

to 10 weeks old) when the ratio is also relatively stable, while those of the 50 gm force were much higher (Figure VI-3). Thus, according to the collagen type III ratio profiles, it appears that light forces tend to initiate a more physiologic response than heavy forces. Moreover, the collagen type III ratio profile of animals subjected to 5 gm of tensile force is almost the same as that obtained from sham-operated animals, suggesting that if the suture is being expanded very slowly with a light force, it is probably not much more traumatic than the surgical procedure itself. In the age study of the mouse interparietal suture (Chapter V), the ratio of the collagen type III eventually drops to zero at about 11 weeks of age, indicating suture maturation. The long term effect of tensile stress should be studied to see when or if the ratio will eventually drop to zero indicating completion of remodelling.

According to the developed fluorographs, radioactivity associated with collagen bands of the higher forces are much more intense than those of the lower forces after the same period of force application (data not shown). However, all sutures were expanded 2 mm and appeared normal to the eye by the end of the experimental period (4 weeks). Thus, with light force, the same objective was achieved with much less synthetic activity, implying a much more efficient remodelling process. When sutures are expanded rapidly, as in the case with heavy forces, a crisis situation in the form of acute trauma results. The primary response of the connective tissue would be to repair in order to restore tissue continuity and subsequently, remodel to obtain proper tis-

sue architecture. This means a gradual replacement of much of the initially rapidly laid down collagen with collagen that is being laid down in a more organized fashion. With a gradual expansion of the suture, much of the original synthetic activities may result in structurally useful collagen, requiring less remodelling to establish the original tissue architecture. A study of the turnover rates of the newly synthesized collagen and the determination of the proportions of newly synthesized collagen being incorporated into mature collagen with reference to tensile stress of different force levels would provide useful information in this respect. The true picture probably is even more complicated since under normal conditions, 10 to 40% of all collagen produced by diploid human fibroblasts is degraded within the cell prior to its secretion (Berg et al., 1978; Bienkowski et al., 1978; Steinmann et al., 1979; Baum et al., 1980).

The changes of collagen type III ratios may be due to a number of possible changes of collagen metabolism, including a differential increase in the synthesis and/or decrease in the degradation of the different phenotypes. Previous investigations into the response of interparietal sutures to mechanical stress indicated a general increase in protein synthesis in collagens solubilized by pepsin digestion (Meikle et al., 1979) and by radioautographic technique (Chiang, 1981). Although synthesis of degrdative enzymes, including collagenase, was also increased, they were inactivated by complexing with inhibitors (Meikle et al., 1980), thus preventing hydrolysis of newly synthesized peptides. Also, the reparative response of wound healing begins with

the appearance of connective tissue cells which deposit a fine reticular network of fibrils derived from type III collagen molecules. Later, larger fibres derived from type I collagen molecules predominate, also with increasing number of fibroblasts (Gay and Miller, 1978). Thus, it is likely that an increase in type III ratio in the present case is due to a proportionally greater increase of type III synthesis than type I.

From the studies of human gingiva, there appears to be functionally distinct subpopulations of fibroblasts such that some fibroblasts produce both type I and type III collagen, while others produce only type I (Engel et al., 1980; Hassell and Stanek, 1983).

The increase in collagen synthesis can be a result of increase in cell function and/or increase in the number of functioning cells, while the changes in type III ratios can be a result of change in cell function or a shift in cell population. In stressed sutures, changes in collagen phenotype synthesis were found to be significant within 4 hours (Chiang, 1981) and within 6 hours for light forces in the present study. This early response is probably due to a change in cellular synthetic function of existing cells as transcriptional and translational changes can be achieved quickly. A change in cell population may then follow by either mobilizing mesenchymal progenitor cells and/or increased mitosis of existing cells. Yee (1979) noticed an increase in mitotic cells in periodontal ligament subjected to stress. However, the cytoplasm of many of these mitotic cells was also dominated by rough endoplasmic reticulum. In addition, intracel-

lular collagen containing vesicles were also present in the cytoplasm of many mitotic cells. This tends to suggest that at least part of the increase in functional cells is due to proliferation of differentiated fibroblasts. It has also been demonstrated that most fibroblast-like cells from explants of periodontal ligament originate from a specific variety of blood vessels (Brunnette et al., 1976). This is also true for cells that divide in response to wounding of the periodontal ligament in vivo (Gould et al., 1977). Thus, local differentiated cells and progenitor cells with haematopoietic origin are both responsible for the increase in functional cells in response to changes in local functional demands.

However, the regulatory and control mechanisms responsible for these changes in cellular activities are still unknown. Nevertheless, collagen types may still serve as important parameters of such changes. If the regulators which mediate such shifts in the cell population, or those responsible for transcriptional, translational and post-translational control during collagen synthesis can be identified, then better understanding may be gained into the remodelling process in the fibrous joints under normal physiological conditions, as well as when subjected to therapeutic mechanical stress. It is now possible to obtain antibodies to specific collagen types (Bornstein and Sage, 1980). These can be used to label and localize collagen synthesizing cells with the subsequent identification of cell populations active in remodelling. After the identification and isolation of these cell populations, cell culture techniques can be utilized

for the study of control mechanisms and effects of various suggested biologic mediators: c-AMP, parathyroid hormone, prostaglandins, vitamin D, etc..

Type V collagen is present in most connective tissues along with types I and III (Rhodes and Miller, 1978). Type V collagen appears to be involved in many important biological processes such as platelet aggregation, epithelial cell migration, substrate attachment and binding of other interstitial collagen fibrils (Narayanan and Page, 1983b). Also, abnormal amounts of type V collagen are present in many diseases such as atherosclerosis and chronic inflammation (Narayanan and Page, 1983b). The choice of collagen type III ratio as the parameter for study in the present investigation is because of the relatively large quantities that can be extracted for study, and because much information about this ratio is available from previous studies, especially in other bone induction systems. However, the possible involvement of other collagen types as well as other extracellular components in the remodelling fibrous joints should not be overlooked: as the synthesis and maintenance of connective tissue ground substance are also functions of fibroblasts. Involvement of ground substance in a variety of metabolic activities has been reported: in formation and orientation of collagen fibres (Mathews, 1965; Toole and Lowther, 1968; Plecash, 1972; Johnson, 1981), in osteogenic activity during suture growth (Persson, 1973), in the transport of tropocollagen (Hay, 1978; Olson and Low, 1980), and in the possible control of cytodifferentiation of mesenchymal cells (Silbert, 1978). However, no information is available concerning ground

substance metabolism during sutural or periodontal ligament remodelling.

The present model system is equally suitable for the study of the effects of compressive stress on fibrous joints, though only tensile stress effects were studied in the present investigation. However, the effect of mechanical stress upon cementum and root resorption cannot be studied with the present model system, due to the fundamental structural difference between the sutural and periodontal fibrous joints. Thus, study of cranial sutures alone could not reveal the total picture of periodontal remodelling in response to orthodontic movement of teeth. Also the differences in effects of intermittant and continuous force systems should be explored.

CONCLUSIONS

1). The collagen type III ratio profiles of the sham-operated and experimental animals showed the same general outline: a rapid increase followed by an almost equally rapid decrease and then stabilized at a level higher than that of the age controls.

2). These profiles are remarkably similar to that of the suture during the first four weeks of life when rapid growth and development are taking place.

3). Tensile stress without causing excessive tissue damage is best able to initiate the increase in type III ratio and stimulate the remodelling process.

4). The collagen type III ratio profile due to the lightest force (5 gm) is very similar to that during rapid growth and development of the suture.

5). It appears that light forces tend to initiate a more physiological response and a more efficient remodelling process than heavy forces.

BIBLIOGRAPHY

BIBLIOGRAPHY

ABE, S., STEINMANN, B.U., WAHL, L.M. and MARTIN, G.R.: High cell density alters the ratio of type III to I collagen synthesis by fibroblasts. Nature 279: 442-444, 1979.

AL-BAREEDI, S., FORBES, D.P., PERRY, H.T. and KAMINSKI, E.J.: Secondary cartilage formation in the midpalatal suture. J. Dent. Res. 63: Abstract 933, 1984.

ALEXANDER, S.A. and SWERDLOFF, M.: Mucopolysaccharidase activity during human deciduous root resorption. Arch. Oral Biol. 24: 735-738, 1979.

ANDERSON, A.A.: The protein matrixes of the teeth and periodontium in hamsters: A tritiated proline study. J. Dent. Res. 46: 67-78, 1967.

ARMITAGE, G.C.: Cementum. In: Orban's Oral Histology and Embryology. Bhaskar, S.N. (Ed.), C.V. Mosby Co., St. Louis, pp. 180-203, 1980.

AUMAILLEY, M., KRIEG, T., DESSAU, W., MULLER, P.K., TIMPL, R. and BRICAUD, H.: Biochemical and immunological studies of fibroblasts derived from a patient with Ehlers-Danlos Syndrome type IV demonstrates reduced type III collagen synthesis. Arch. Dermat. Res. 269: 169-177, 1980.

BADELL, M.D.: An evaluation of extra oral combined high-pull traction and cervical traction to the maxilla. Am. J. Orthodont. 69: 431-446, 1976.

BAER, M.: Patterns of growth of the skull as revealed by vital staining. Hum. Biol. 26: 80-126, 1954.

BAILEY, A.J. and ROBINS, S.P.: Current topics in the biosynthesis, structure and function of collagen. Scient. Orig, Oxf. 63: 419-444, 1976.

BALLARD, J.B. and BUTLER, W.T.: Proteins of the periodontium. Biochemical studies on the collagen and non-collagenous proteins of human gingivae. J. Oral Path. 3: 176-184, 1974.

BARBANELL, R.L., LIAN, J.B. and KEITH, D.A.: Structural proteins of connective tissues. In: Textbook of Oral Biology. Shaw, J.H., Sweeney, E.A., Cappuccino, C.C. and Meller, S.M. (Eds.), W.B. Saunders, Philadelphia, pp. 419-452, 1978.

BARNES, M.J., MORTON, L.F., BENNETT, R.C., BAILEY, A.J. and SIMS, T.J.: Presence of type III collagen in guinea pig dermal

sear. Biochem. J. 157: 263-266, 1976.

BASSETT, C.A.L.: Biological significance of piezoelectricity. Calcif. Tissue Res. 1: 252-272, 1968.

BASSETT, C.A.L., PAWLUK, R. and BECKER, R.O.: Effects of electric current on bone in vivo. Nature 204: 652-654, 1964.

BAUM, B.J., MOSS, J., BREUL, S.D., BERG, R.A. and CRYSTAL, R.G.: Effect of cyclic AMP on the intracellular degradation of newly synthesized collagen. J. Biol. Chem. 255: 2843-2847, 1980.

BAUMHAMMERS, A. and STALLARD, R.E.: ^{35}S-sulphate utilization and turnover by the connective tissue of the periodontium. J. Periodont. Res. 3: 187-193, 1968.

BAUMRIND, S.: A reconsideration of the property of the pressure-tension hypothesis. Am. J. Orthodont. 55: 12-21, 1969.

BAUMRIND, S. and BUCK, D.L.: Rate changes in cell replication and protein synthesis in the periodontal ligament incident to tooth movement. Am. J. Orthodont. 57: 109-131, 1970.

BEERTSEN, W.: Remodelling of collagen fibres in the periodontal ligament and the supra-alveolar region. Angle Orthodont. 49: 218-224, 1979.

BEERTSEN, W. and EVERTS, V.: The site of remodelling of collagen in the periodontal ligament of the mouse incisor. Anat. Rec. 189: 479-498, 1977.

BEGG, P.R. and KESLING, P.C.: Begg Orthodontic Theory and Technique. W.B. Saunders Co., Philadelphia, Chap. 1 Preamble, Chap. 2 Correct Occlusion. The Basis of Orthodontics, pp. 1-50, 1977.

BERG, R.A., SCHWARTZ, M.L. and CRYSTAL, R.G.: Regulation of collagen production in cultures of human fibroblasts by the modulation of collagen degradation. Fed. Proc. 38: Abstract 3014, 1978.

BERNICK, S.: Organization of the periodontal membrane fibers of the developing molars of rats. Arch. Oral Biol. 2: 57-63, 1960.

BIEN, S.M.: Fluid dynamic mechanisms which regulate tooth movement. Adv. Oral Biol. 2: 173-202, 1966.

BIENKOWSKI, R.S., BAUM, B.J. and CRYSTAL, R.G.: Fibroblasts degrade newly synthesized collagen within the cell before secretion. Nature 276: 413-416, 1978.

BINDERMAN, I. and COX, J.C.: Effect of mechanical stress on cultured periosteum cells: Stimulation of DNA synthesis. J. Dent. Res. 56B: Abstract 86, 1977.

BIRKEDAL-HANSEN, H., BUTLER, W.T. and TAYLOR, R.E.: Proteins of the periodontium. Characterization of the insoluble collagens of bovine dental cementum. Calcif. Tissue Res. 23: 39-44, 1977.

BJORK, A.: Sutural growth of the upper face studied by the implant method. Acta Odontol. Scand. 24: 109-127, 1966.

BLOORE, J.A., FURSTMAN, L. and BERNICK, S.: Postnatal development of the rat palate. Am. J. Orthodont. 56: 505-515, 1969.

BONNER, W.M. and LASKEY, R.A.: A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. Europ. J. Biochem. 46: 83-88, 1974.

BORNSTEIN, P. and SAGE, H.: Structurally distinct collagen types. Ann. Rev. Biochem. 49: 957-1003, 1980.

BORNSTEIN, P. and TRAUB, W.: The structure proteins. In: The Proteins. 3rd ed., Vol. 4, Neurath, H. and Hill, R. (Eds.), Plenum Press, New York, pp. 163-273, 1979.

BOURRET, L.A. and RODAN, G.A.: The role of calcium in the inhibition of cAMP accumulation in epiphyseal cartilage cells exposed to physiological pressure. J. Cell Physiol. 88: 353-362, 1976.

BRANDT, H.C., SHAPIRO, P.A. and KOKICH, V.G.: Experimental and post-experimental effects of posteriorly directed extra-oral traction in adult Macaca fosciculari s. Am. J. Orthodont. 75: 301-317, 1979.

BROSSMAN, R.E., BENNETT, C.G. and MEROW, W.W.: Facioskeletal remodelling resulting from rapid palatal expansion in the monkey (Macaca cynomolgus). Arch. Oral Biol. 18: 987-994, 1973.

BRUNNETTE, D.M., MELCHER, A.H. and MOE, H.K.: Culture and origin of epithelium-like and fibroblast-like cells from porcine periodontal ligament explants and cell suspension. Arch. Oral Biol. 21: 393-400, 1976.

BUCK, D.L. and CHURCH, D.H.: A histological study of human tooth movement. Am. J. Orthodont. 62: 507-516, 1972.

BURGESON, R.E., EL ADLI, F.A., KAITILA, I.I. and HOLLISTER, D.W.: Fetal membrane collagens: identification of two new collagen alpha chains. Proc. Natl. Acad. Sci. U.S.A. 73: 2579-2583, 1976.

BUTLER, N.T., BIRKEDAL-HANSEN, J., BEAGLE, N.E., TAYLOR, R.E. and CHUNG, E.: Proteins of the periodontium. Identification of collagens with the [alpha 1 (I)2]alpha 2 and [alpha 1 (III)]3 structures in bovine periodontal ligament. J. Biol. Chem. 250: 8907-8912, 1975.

CARNEIRO, J. and FAVA De MORAES, F.: Radioautographic visualization of collagen metabolism in the periodontal tissues of the mouse. Arch. Oral Biol. 10: 833-845, 1965.

CARNEIRO, J. and LEBLOND, C.: Suitability of collagenase treatment for the radioautographic identification of newly synthesized collagen labelled with ^{3}H-glycine or ^{3}H-proline. J. Histochem. Cytochem. 14: 334-344, 1966.

CHIANG, S.K.: The effect of orthopaedic stress on the collagen and non-collagenous protein synthesis in age-selected white male mice: a radioautographic and biochemical study. M.Sc. Thesis, University of Manitoba, Winnipeg, 1981.

CHUNG, E. and MILLER, E.J.: Collagen polymorphism: Characterization of molecules with the chain composition [alpha 1 (III)]3 in human tissues. Science 183: 1200-1201, 1974.

CIANCIO, S.C., NEIDER, M.D. and HAZEN, S.P.: The principal fibers of the periodontal ligament. Periodontics 5: 76-81, 1967.

CLAYCOMB, C.K., SUMMERS, G.W. and DVORAK, E.M.: Oral collagen biosynthesis in the guinea pig. J. Periodont. Res. 2: 115-120, 1967.

CLEALL, J.F.: Growth of the palate and maxillary dental arch. J. Dent. Res. 53: 1226-1234, 1974.

CLEALL, J.H., BAYNE, D.I., POSEN, J.M. and SUBTELNY, J.D.: Expansion of the midpalatal suture in the monkey. Angle Orthodont. 35: 23-35, 1965.

CLEALL, J.F., JACOBSON, S.H., CHEBIB, F.S. and BARKER, S.: Growth of the craniofacial complex in the rat. Am. J. Orthodont. 60: 368-381, 1971.

COHN, S.A.: Disease atrophy of the periodontium in mice following partial loss of function. Arch. Oral Biol. 11: 95-105, 1966.

COOLIDGE, E.D.: The thickness of the human periodontal membrane. J. Am. Dent. Assoc. 24: 1260-1270, 1937.

CRUMLEY, P.J.: Collagen formation in the normal and stressed periodontium. Periodontics 2: 53-61, 1964.

CURRAN, R.C. and COLLINS, D.H.: Mucopolysaccharides in fields of intramembranous ossification in man. J. Path. Bact. 74: 207-214, 1957.

DAVIDOVITCH, Z., FINKELSON, M.D., STEIGMAN, S., SHANFELD, J.L., MONTGOMERY, P.C. and KOROSTOFF, E.: Electric currents, bone remodelling and orthodontic tooth movement. I. The effect of electric currents on periodontal cyclic nucleotides. Am. J. Orthodont. 77: 14-32, 1980a.

DAVIDOVITCH, Z., FINKELSON, M.D., STEIGMAN, S., SHANFELD, J.L., MONTGOMERY, P.C. and KOROSTOFF, E.: Electric currents, bone remodelling and orthodontic tooth movement. II. Increase in rate of tooth movement and periodontal cyclic nucleotide levels by combined force and electric current. Am. J. Orthodont. 77: 33-47, 1980b.

DAVIDOVITCH, Z., KOROSTOFF, E., FINKELSON, M., STEIGMAN, S., SHANFELD, J.L. and MONTGOMERY, P.C.: Cyclic nucleotides in periodontal tissues following simultaneous electric-orthodontic treatment. J. Dent. Res. 58A: 402 (Abstract), 1979.

DAVIDOVITCH, Z., KOROSTOFF, E., SHANFELD, J., MONTGOMERY, P. and FINKELSON, M.: Effects of minute electric currents on cyclic nucleotides of cat periodontal tissues. J. Dent. Res. 57A: 348 (Abstract), 1978a.

DAVIDOVITCH, Z., MONTGOMERY, P.C., ECKERDAL, O. and GUSTAFSON, G.T.: Demonstration of cyclic AMP in bone cells by immuno-histochemical methods. Calcif. Tissue Res. 19: 305-315, 1976a.

DAVIDOVITCH, Z., MONTGOMERY, P.C., ECKERDAL, O. and GUSTAFSON, G.T.: Cellular localization of cyclic AMP in periodontal tissues during experimental tooth movement in cats. Calcif. Tissue Res. 19: 317-329, 1976b.

DAVIDOVITCH, Z., MONTGOMERY, P.C. and SHANFELD, J.L.: Guanosine 3',5'-monophosphate in bone: Microscopic visualization by an immuno-histochemical technique. Calcif. Tissue Res. 24: 73-79, 1977.

DAVIDOVITCH, Z., MONTGOMERY, P.C., YOST, R.W. and SHANFELD, J.L.: Immuno-histochemical localization of cyclic nucleotides in the periodontium: Mechanically-stressed cells in vivo. Anat. Res. 192: 351-361, 1978.

DAVIDOVITCH, Z., MUSICH, D. and DOYLE, M.: Hormonal effects on orthodontic tooth movement in cats - a pilot study. Am. J. Orthodont. 62: 95-96, 1972.

DAVIDOVITCH, Z. and SHANFELD, J.L.: Cyclic AMP levels in alveolar

bone of orthodontically treated cats. Arch. Oral Biol. 20: 567-574, 1975.

DE ANGELIS, V.: Autoradiographic investigation of calvarial growth in the rat. Am. J. Anat. 123: 359-367, 1968.

DEPORTER, D.A. and TEN CATE, A.R.: Time structural localization of acid and alkaline phosphatase in collagen-containing vesicles of fibroblasts. J. Anat. 114: 457-461, 1973.

DIAZ, E.: Periodontal ligament collagen response to tooth movement: histochemical and autoradiographic reactions. Am. J. Orthodont. 73: 443-457, 1978.

DIXON, A.D.: Studies of the growth of the upper facial skeleton using radioactive calcium^{45}. J. Dent. Res. 40: 204-216, 1961.

DROSCHL, H.: The effect of heavy orthopaedic forces on the maxilla in the growing Saimiri Scillreus (squirrel monkey). Am. J. Orthodont. 63: 449-461, 1973.

DROSCHL, H.: The effect of heavy orthopaedic forces on the suture of the facial bones. Angle Orthodont. 45: 26-33, 1975.

DUNCAN, G.W.: The role of prostaglandins in connective tissue remodelling during orthodontic tooth movement. M.Sc. Thesis, University of Manitoba, Winnipeg, 1982.

DUNCAN, G.W., YEN, E.H.K., PRITCHARD, E.T. and SUGA, D.M.: Collagen and prostaglandin synthesis in force-stressed periodontal ligament in vitro. J. Dent. Res. 63: 665-669, 1984.

ECCLES, J.D.: Studies on the development of the periodontal membrane: The principal fibres of the molar teeth. Dent. Pract. Dent. Rec. 10: 31-35, 1959.

EIGEN, M. and DeMAEYER, L.: Self-dissociation and protonic charge transport in water and ice. Proc. Roy. Soc. London A247: 505-533, 1958.

ELDER, J.R. and TUENGE, R.H.: Cephalometric and histologic changes produced by extraoral high-pull traction to the maxilla in Macaca mulatta. Am. J. Orthodont. 66: 599-617, 1974.

ENGDAHL, E., RITSILA, V. and VOOSTROMER, L.: Growth potential of cranial suture bone autographs, Part I and II. Scand. J. Plast. Reconstr. Surg. 12: 119-129, 1978.

ENGEL, L.D., SCHROEDER, H.E., GAY, R. and CLAQETT, J.: Fine structure of cultured human gingival fibroblasts and demonstration of simultaneous synthesis of types I and III

collagen. Arch. Oral Biol. 25: 283-296, 1980.

ENLOW, D.H.: The Human Face. Harper and Row, New York, 1968.

ENLOW, D.H.: Handbook of Facial Growth. W.B. Saunders Co., Philadelphia, Chap. 2 Introductory Concepts of the Growth Process, p. 38, 1982.

EPSTEIN, E.H.: [alpha 1 (III)]3 Human skin collagen. Release by pepsin digestion and preponderance in fetal life. J. Biol. Chem. 249: 3225-3231, 1974.

EPSTEIN, E.J. Jr. and MUNDERLOCH, W.H.: Isolation and characterization of CNBr peptides of human [alpha 1 (III)]3 collagen and tissue distribution of [alpha 1 (I)]2 alpha 2 and [alpha 1 (III)]3 collagens. J. Biol. Chem. 250: 9304-9312, 1975.

EVERTS, V. and BEERTSEN, W.: Identity of a population of progenitor cells in gingival connective tissue of the mouse incisor. Anat. Rec. 192: 319-324, 1978.

EYRE, D.R. and MUIR, H.: Type III collagen: A major constituent of rheumatoid and normal human synovial membrane. Conn. Tissue Res. 4: 11-16, 1975.

FAIRBANKS, G., STECK, T.L. and WALLACH, D.F.M.H.: Electrophoretic analysis of the major polypeptides of human erythrocyte membrane. Biochem. 10: 2606-2617, 1971.

FENG, J., MELCHER, A.H., BRUNETTE, D.M. and MOE, H.R.: Determination of L-ascorbic acid levels in culture medium: Concentrations in commercial media and maintenance of levels under conditions of organ culture. In Vitro 13: 91-99, 1977.

FIRSCHEIN, H.E.: Comparative kinetics of collagen biosynthesis in various bones. Comp. Biochem. Physiol. 22: 601-606, 1967.

FRIEDE, H.: A histological and enzyme-histochemical study of growth sites of the premaxilla in human foetuses and neonates. Arch. Oral Biol. 20: 809-814, 1975.

FUKADA, E. and YUSADA, I.: On the piezo-electric effect of bone. J. Phys. Soc. Japan 12: 1158-1162, 1957.

GANS, B.J. and SARNAT, B.G.: Sutural facial growth of the Macaca rhesus monkey: A gross and serial roentgenographic study by means of metallic implants. Am. J. Orthodont. 37: 827-841, 1951.

GARANT, P.R.: Collagen resorption by fibroblasts. A theory of fibroblastic maintenance of periodontal ligament. J. Periodontol. 47: 380-390, 1976.

GAY, S. and MILLER, E.J.: Collagen in the Physiology and Pathology of Connective Tissue. Fischer Verlag, New York, 1978.

GAY, S., VILJANTO, J., RAEKALLIC, J. and PENTTINEN, R.: Collagen types in early phases of wound healing in children. Acta Chir. Scand. 144: 205-211, 1978.

GIANELLY, A.A.: Force induced changes in the vascularity of the periodontal ligament. Am. J. Orthodont. 55: 5-11, 1969

GIANELLY, A.A. and SCHMUR, R.M.: The use of parathyroid hormone to assist orthodontic tooth movement. Am. J. Orthodont. 55: 305 (Abstract), 1969.

GILMAN, T.: On some aspects of collagen formation in localized repair and in diffuse fibrotic reactions to injury. In: Treatise on Collagen. 2. Part B, Gould, B. (Ed.), Academic Press, New York, pp. 332-407, 1968.

GLASSTONE, S.: The development of tooth germs in vitro. J. Anat. 70: 260-266, 1936.

GLASSTONE, S.: A comparative study of the development in vitro and in vivo of rat and rabbit molars. Proc. Roy. Soc. B., 126: 315-330, 1938.

GLASSTONE, S.: The development of halved tooth germs. J. Anat. 86: 12-15, 1952.

GLASSTONE, S.: The development of tooth germs on the chick chorioallantois. J. Anat. 88: 392-399, 1954.

GLASSTONE, S.: Cultivation of mouse tooth germs in a chemically defined protein-free medium. Arch. Oral Biol. 9: 27-30, 1964.

GOLDHABER, P.: The effect of hyperoxia on bone resorption in tissue culture. Arch. Path. 66: 634-641, 1958.

GOLDHABER, P: Oxygen dependent bone resorption in tissue culture. In: The Parathyroids, Greep, R.O. and Talmage, R.V. (Eds.), Chas. C. Chomas, Springfield, pp. 243-254, 1961.

GOLDHABER, P.: Remodelling of bone tissue. J. Dent. Res. 45: 490-499, 1966.

GOLDMAN, H.M.: Discussion of connective tissues: Periodontology. J. Dent. Res. 41: 230-234, 1962.

GOULD, T.R.L., MELCHER, A.H. and BRUNETTE, D.M.: Location of progenitor cells in periodontal ligament of mouse molar stimulated by wounding. Anat. Rec. 188: 133-142, 1977.

GRANT, D.A. and BERNICK, S.: Formation of the periodontal ligament. J. Periodont. 43: 17-25, 1972. 17-25, 1972.

GRIFFITHS, D.L., FURSTMAN, L. and BERNICK, S.: Postnatal development of the mouse palate. Am. J. Orthodont. 53: 757-768, 1967.

HAAS, A.J.: Palatal expansion: Just the beginning of dentofacial orthopaedics. Am. J. Orthodont. 57: 219-235, 1970.

HALL, B.K.: Development and Cellular Skeletal Biology. Academic Press, New York, Chap. 6 Progenitor Cells and their Differentiation, pp. 107-147, 1978a.

HALL, B.K.: Development and Cellular Skeletal Biology. Academic Press, New York, Chap. 9 Initiation of Skeletal Growth, pp. 224-226, 1978b.

HANCE, A.J. and CRYSTAL, R.G.: Rigid control of synthesis of collagen types I and III by cells in culture. Nature 268: 152-154, 1977.

HANG, S.L., POLSKY-CYNKIN, R. and LAVINE, L.: Stimulation of prostaglandin biosynthesis by vasoactive substances in methylcholanthrene-transformed mouse BALB/3T3. J. Biol. Chem. 251: 776-780, 1976.

HARRY, M.R. and SIMS, M.R.: Root resorption in bicuspid intrusion: A scanning electronic microscopic study. Angle Orthodont. 52 (3): 235-258, 1982.

HASSELL, T.J. and STANEK III, E.J.: Evidence that healthy human gingiva contains functionally heterogenous fibroblast subpopulations. Arch. Oral Biol. 28: 617-625, 1983.

HAY, E.D.: Fine structure of embryonic matrices and their relation to the cell surface in ruthenium red-fixed tissues. Growth 42: 399-423, 1978.

HAY, M.F.: The development in vivo and in vitro of the lower incisor and molars of the mouse. Arch. Oral Biol. 3: 86-109, 1961.

HERRING, S.W.: Sutures - a tool in functional cranial analysis. Acta Anat. 83: 222-247, 1972.

HICKORY, W.B.: In vitro cellular response to cranial suture to tensile force. M.Sc. Thesis, University of Connecticut, Farmington, 1982.

HINDLE, M.O.: The intermediate plexus of the periodontal membrane. In: The Mechanisms of Tooth Support. A Symposium. Anderson, D.J., Eastol, J.E., Melcher, A.H. and Picton,

D.C.A. (Eds.), Wright, Bristol, pp. 66-71, 1967.

HINRICHSEN, G.J. and STOREY, E.: The effect of force on bone and bones. Angle Orthodont. 38: 155-165, 1968.

HONG, Y.C., YEN, P.K.J. and SHAW, J.H.: An analysis of the growth of the cranial vault in rabbits by vital staining with lead acetate. Calcif. Tissue Res. 2: 271-285, 1968.

HOYTE, D.A.N.: Mechanisms of growth in the cranial vault and base. J. Dent. Res. 50: 1447-1461, 1971.

HUNT, A.M.: A description of the molar teeth and investing tissues of normal guinea pigs. J. Dent. Res. 38: 216-231, 1959.

ISAACSON, R., WOOD, J. and INGRAM, A.: Forces produced by rapid maxillary expansion. Angle Orthodont. 34: 256-270, 1964.

ISOTUPA, K., KOSKI, K. and MAKINEN, L.: Changing architecture of growing cranial bones at sutures as revealed by vital staining with Alizarin red S in the rabbit. Am. J. Phys. Anthropol. 23: 19-22, 1965.

JACKSON, H.J. and HEININGER, J.A.: Proline recycling during collagen metabolism as determined by concurrent $^{18}O2$ and ^{3}H-labelling. Biochim. Biophys. Acta 381: 359-366, 1975.

JACKSON, G., KOKICH, V. and SHIPIRO, R.: Experimental and post-experiment response to anteriorly directed extra-oral force in young Macaca nemestrina. Am. J. Orthodont. 75: 318-333, 1979.

JAHN, T.L.: A theory of electronic conduction through membranes and of active transport of ions, based on redox transmembrane potentials. J. Theoret. Biol. 2: 129-138, 1962.

JAHN, T.L.: A possible mechanism for the effect of electrical potentials on apatite formation in bone. Clin. Orthop. 56: 261-273, 1968.

JENSEN, J.L. and TOTO, P.D.: Radioactive labeling index of the periodontal ligament in aging rats. J. Dent. Res. 47: 149-153, 1968.

JIMINEZ, S.A., BASHEY, R.I., BENDITT, M. and YANKOWSKI, R.: Identification of collagen alpha 1 (I) trimer in embryonic chick tendons and calvaria. Biochem. Biophys. Res. Comm. 78: 1354-1361, 1977.

JOHNSON, R.B.: Development of transalveolar fibres in the mouse periodontium. Ph.D. Thesis, University of North Dakota, Grand Forks, 1981.

KAMATA, M.: Effect of parathyroid hormone on tooth movement in rats. Bull. Tokyo Med. Dent. Univ. 19: 411-425, 1972.

KAMBARA, T.: Dentofacial changes produced by extraoral forward force in the Macaca irus. Am. J. Orthodont. 71: 249-277, 1977.

KAMEYAMA, Y.: An autoradiographic investigation of the developing rat periodontal membrane. Arch. Oral Biol. 18: 473-480, 1973.

KAMEYAMA, Y.: Autoradiographic study of ^{3}H-proline incorporation by rat periodontal ligament, gingival connective tissue and dental pulp. J. Periodont. Res. 10: 98-102, 1975.

KAO, K.T., HILKER, D.M. and McGAVACK, T.H.: Connective tissue IV. Synthesis and turnover of proteins in tissues of rats. Proc. Soc. Exp. Biol. Med. 106: 121-124, 1961.

KARDOS, T.B. and SIMPSON, L.O.: A new periodontal membrane biology based upon thixotropic concepts. Am. J. Orthodont. 77: 508-515, 1980.

KHOUW, F.E. and GOLDHABER, P.: Changes in vasculature of the periodontium associated with tooth movement in the Rhesus monkey and dog. Arch. Oral Biol. 15: 1125-1132, 1970.

KIVIRIKKO, K.I. and RISTELI, J.: Biosynthesis of collagen and its alterations in pathological states. Med. Biol. 54: 159-186, 1976.

KOCH, W.E.: In vitro development of tooth rudiments of embryonic mice. Anat. Rec. 152: 513-524, 1965.

KOCH, W.E.: In vitro differentiation of tooth rudiments of embryonic mice. I. Transfilter interaction of embryonic incisor tissues. J. Exp. Zoo. 165: 155-170, 1967.

KOKICH, V.G.: Age changes in the human frontozygomatic suture from 20 to 95 years. Am. J. Orthodont. 69: 411-430, 1976.

KOLLAR, E.J.: Tooth development and dental patterning. In: Morphogenesis and Pattern Formation. Connelly, T.G., Brinkley, L.L. and Carlson, B.M. (Eds.), Raven Press, New York, 1981.

KOLLAR, E.J. and FISHER, C.: Tooth induction in chick epithelium: expression of quiescent genes for enamel synthesis. Science 207: 993-995, 1980.

KOSKINEN, L., ISOTUPA, K. and KOSKI, K.: A note on craniofacial sutural growth. Am. J. Phys. Anthropol. 45: 511-516, 1976.

LAEMMLI, U.K.: Cleavage of structural proteins during the assembly of the head of the bacteriophage. Nature 227: 680-685, 1970.

LATHAM, R.A.: The development, structure and growth pattern of the human mid-palatal suture. J. Anat. 108: 31-41, 1971.

LEBLOND, C.P., MESSIER, B. and KOPRIWA, B.: Thymidine ^{3}H as a tool for the investigation of the renewal of cell populations. Lab. Invest. 8: 296-308, 1959.

LEBLOND, C.P., WILKINSON, G.W., BILANGER, L.F. and ROBICHON, J.: Radioautographic visualization of bone formation in the rat. Am. J. Anat. 86: 289-342, 1950.

LEFKOWITZ, W. and SWAYNE, P.: The cultivation of tooth germs in an embryo extract-free medium. J. Dent. Res. 35: 440-445, 1956a.

LEFKOWITZ, W. and SWAYNE, P.: In vitro cultivation of rat tooth germs. J. Dent. Res. 35: 523-532, 1956b.

LEFKOWITZ, W. and SWAYNE, P.: Normal development of tooth buds cultured in vitro. J. Dent. Res. 37: 1100-1114, 1958.

LEUNG, D.V.M., GLAGOV, S. and MATHEWS, M.B.: Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. Science 191: 475-477, 1976.

LEUNG, D.V.M., GLAGOV, S. and MATHEWS, M.B.: A new in vitro system for studying cell response to mechanical stimulation. Different effects of cyclic stretching and agitation on smooth muscle cell biosynthesis. Exp. Cell Res. 109: 285-298, 1977.

LEWIN, E. and IRWING, J.: An autoradiographic investigation of bone remodelling in the rat calvarium in organ culture. Arch. Oral Biol. 15: 769-776, 1970.

LIMEBACK, H.F., SODEK, J. and BRUNNETTE, D.: Nature of collagens synthesized by monkey periodontal ligament fibroblasts in vitro. Biochem. J. 170: 63-71, 1978.

LINGE, L.: Tissue reactions incident to widening of facial sutures. Trans. Europ. Orthodont. Soc. 487-497, 1972.

LINGE, L.: Tissue reactions in facial sutures subsequent to external mechanical influences. In: Factors Affecting the Growth of the Midface. McNamara, J. (Ed.), Centre for Human Growth and Development, University of Michigan, Ann Arbor, pp. 251-275, 1976.

LISTGARTEN, M.A.: Intracellular collagen fibrils in the

periodontal ligament of the mouse, rat, hamster, guinea pig and rabbit. J. Periodont. Res. 8: 335-342, 1973.

MACAPANPAN, L.C., MEYER, J. and WEINMANN, J.P.: Mitotic activity of fibroblasts after damage of the periodontal membrane of rat molars. J. Periodontol. 25: 105-112, 1954.

MADRI, J.A. and FURTHMAYR, H.: Isolation and tissue localization of type AB2 collagen from normal lung parenchyma. Am. J. Pathol. 94: 323-331, 1979.

MASSLER, M. and SCHOUR, I.: The growth pattern of the cranial vault in the albino rat as measured by vital staining with Alizarin red S. Anat. Rec. 110: 83-101, 1951.

MATHEWS, M.B.: The interaction of collagen and acid mucopolysaccharides. Biochem. J. 96: 710-715, 1965.

MEIKLE, M.C., HEATH, J.K., HEMBRY, R.M. and REYNOLDS, J.J.: Rabbit cranial suture fibroblasts under tension express a different collagen phenotype. Arch. Oral Biol. 27: 609-613, 1982.

MEIKLE, M.C., REYNOLDS, J.J., SELLERS, A. and DINGLE, J.T.: Rabbit cranial sutures in vitro. A new experimental model for studying the response of fibrous joints to mechanical stress. Calcif. Tissue Int. 28: 137-144, 1979.

MEIKLE, M.C., SELLERS, A. and REYNOLDS, J.J.: Effect of tensile mechanical stress on synthesis of metalloproteinases by rabbit coronal sutures in vitro. Calcif. Tissue Int. 30: 77-82, 1980.

MELCHER, A.H.: Remodelling of the periodontal ligament during eruption of the rat incisor. Arch. Oral Biol. 12: 1649-1652, 1967.

MELCHER, A.H.: On the repair potential of periodontal tissues. J. Periodontol. 47: 256-260, 1976.

MELCHER, A.H.: Periodontal ligament. In: Orban's Oral Histology and Embryology. Bhaskar, S.N. (Ed.), C.V. Mosby Co., St. Louis, pp. 204-239, 1980.

MELCHER, A.H. and CORREIA, M.A.: Remodelling of periodontal ligament in erupting molars of mature rats. J. Periodont. Res. 6: 118-125, 1971.

MELCHER, A.H. and TURNBULL, R.S.: Organ culture in studies of the periodontium. In: Organ Culture in Biomedical Research. Balls, M. and Monnickendam, M. (Eds.), Cambridge University Press, Cambridge, pp. 149-163, 1976.

MELSEN, B.: A histological study of the influence of sutural morphology and skeletal maturation on rapid palatal expansion in children. Trans. Europ. Orthodont. Soc. 499-507, 1972.

MELSEN, B.: Palatal growth studied on human autopsy material. Am. J. Orthodont. 68: 42-54, 1975.

MESSIER, B. and LEBLOND, C.P.: Cell proliferation and migration as revealed by radioautography after injection of ^{3}H-thymidine into male rats and mice. Am. J. Anat. 106: 247-265, 1960.

MILLER, E.J.: Structural studies of cartilage collagen employing limited cleavage and solubilization with pepsin. Biochemistry 11: 4903-4909, 1972.

MILLER, E.J.: Biochemical characteristics and biological significance of the genetically distinct collagens. Mol. Cell Biochem. 13: 165-192, 1976.

MINOR, R.R.: Collagen metabolism, a comparison of diseases of collagen and diseases affecting collagen. Am. J. Pathol. 98: 227-280, 1980.

MIROUE, M.A. and ROSENBERG, L.: The human facial sutures: a morphologic and histologic study of age changes from 20 to 95 years. M.S.D. Thesis, University of Washington, Seattle, 1975.

MOFFETT, B.C.: Remodelling of the craniofacial articulations by various orthodontic appliances in Rhesus monkey. Trans. Europ. Orthodont. Soc. 1971: 207-216, 1971.

MOFFETT, B.C.: Remodelling of the craniofacial skeleton produced by orthodontic forces. In: Symposia of the Fourth International Congress of Primatology. Vol. 3, S. Karger, Basel, pp. 180-190, 1973.

MOORE, A.: Head growth of the Macaque monkey as revealed by vital staining, embedding and undercalcified sectioning. Am. J. Orthodont. 34: 654-671, 1949.

MOSS, M.L.: Growth of the calvaria in rat, the determination of osseous morphology. Am. J. Anat. 94: 333-362, 1954.

MOSS, M.L.: Experimental alteration of sutural area morphology. Anat. Rec. 127: 569-589, 1957.

MOSS, M.L.: The functional matrix. In: Vistas in Orthodontics. Kraus, B.S. and Reidel, R. (Eds.), Lea and Febiger, Philadelphia, 1962.

MOSS, M.L. and YOUNG, R.W.: A functional approach to craniology.

Am. J. Phys. Anthropol. 18: 281-292, 1960.

MULLER, P.K., KIRSCH, E., GAUSS-MULLER, V. and KRIEG, T.: Some aspects of the modulation and regulation of collagen synthesis in vitro. Mol. Cell. Biochem. 34: 73-85, 1981.

MULLER, P.K., LEMMEN, G., GAY, S. and MEIGEL, W.N.: Disturbance in the regulation of the type of collagen synthesized in a form of osteogenesis imperfecta. Eur. J. Biochem. 59: 97-104, 1975.

MUNKSGAARD, E.C., RHODES, M., MAYNE, R. and BUTLER, W.T.: Collagen synthesis and secretion by rat incisor odontoblasts in organ culture. J. Biochem. 82: 609-617, 1978.

MURRAY, J. and CLEALL, J.F.: Early tissue response to rapid maxillary expansion in the mid-palatal suture of the Rhesus monkey. J. Dent. Res. 50: 1654-1660, 1971.

NANDA, R.: Protraction of maxilla in rhesus monkeys by controlled extraoral forces. Am. J. Orthodont. 74: 121-141, 1978.

NARAYANAN, A.S., ENGEL, L.D. and PAGE, R.C.: The effect of chronic inflammation in the composition of collagen types in human connective tissue. Collagen Rel. Res. 3: 323-334, 1983.

NARAYANAN, A.S. and PAGE, R.C.: Serum modulates collagen types in human gingiva fibroblasts. FEBS Lett. 80: 221-224, 1977.

NARAYANAN, A.S. and PAGE, R.C.: Connective tissues of the periodontium: A summary of current work. Collagen Rel. Res. 3: 33-64, 1983a.

NARAYANAN, A.S. and PAGE, R.C.: Biosynthesis and regulation of type V collagen in diploid human fibroblasts. J. Biol. Chem. 258: 11694-11699, 1983b.

NARAYANAN, A.S., PAGE, R.C. and MEYERS, D.F.: Characterization of collagens of diseased human gingiva. Biochemistry 19: 5037-5043, 1980.

NAVIA, J.M.: Animal Models in Dental Research. The University of Alabama Press, Birmingham, 1977.

NOLAN, J.C., CARDINALE, G.J. and UDENFRIEND, S.: The formations of hydroxyproline in collagen by cells grown in the absence of serum. Acta Biochim. Biophys. 543: 116-122, 1978.

NORTON, L.A., RODAN, G.A. and BOURRET, L.A.: Epiphyseal cartilage cAMP changes produced by electrical and mechanical perturbations. Clin. Orthop. 124: 59-68, 1977.

NOYES, F.B.: The structure of the suture. Angle Orthodont. 4: 123-130, 1934.

OLSEN, G.E. and LOW, F.N.: Relationships among extracellular components in the developing chick aorta. J. Submicroc. Cytol. 12: 507-521, 1980.

OSBORN, J.W. and TEN CATE, A.R.: Advanced Dental Histology. 4th ed., John Wright and Sons, Bristol, Chap. 23 Age Changes in the Dental Tissues, pp. 201-202, 1983a.

OSBORN, J.W. and TEN CATE, A.R.: Advanced Dental Histology. 4th ed., John Wright and Sons, Bristol, Chap. 20 Physiological Tooth Movement, pp. 171-182, 1983b.

PAGE, R.C. and AMMONS, W.F.: Collagen turnover in gingiva and other mature connective tissues of the marmoset. Arch. Oral Biol. 19: 651-658, 1974.

PAGE, R.C. and SCHOEDER, H.E.: Periodontitis in Man and Other Animals. S. Karger, Basel, Chap. 1 Introduction, Chap. 2 Periodontitis in Humans, pp. 1-45; Chap. 4 Discussion, pp. 239-251, 1982.

PARAKKAL, P.F.: Involvement of macrophages in collagen resorption. J. Cell Biol. 41: 345-354, 1969a.

PARAKKAL, P.F.: Role of macrophages in collagen resorption during hair growth cycle. J. Ultrastruct. Res. 29: 210-217, 1969b.

PENTTINNEN, R.P., LICHTENSTEIN, J.R., MARTIN, G.R. and McKUSICK, V.A.: Abnormal collagen metabolism in cultured cells in osteogenesis Imperfecta. Proc. Natl. Acad. Sci. U.S.A. 72: 586-589, 1975.

PERSSON, M.: Structure and growth of facial sutures. Odontologisk Revy. 24: suppl. 26, 1973.

PERSSON, M. and ROY, W.: Suture development and bony fusion in the fetal rabbit palate. Arch. Oral Biol. 24: 283-291, 1979.

PLECASH, J.M.: Associations of acid mucopolysaccharides with normal gingival collagen fibrils in the rat molar periodontium. J. Canad. Dent. Assoc. 7: 246-247, 1972.

POLLACK, S.V.: Wound healing: a review. III. Nutritional factors affecting wound healing J. Dermatol. Surg. Oncol. 5: 8-25, 1979.

POPE, F.M., MARTIN, G.R., LICHTENSTEIN, J.R., PENTTINNEN, R., GERSON, B., ROWE, D.W. and McKUSICK, V.A.: Patients with

Ehlers Danlos Syndrome type IV lack type III collagen. Proc. Natl. Acad. Sci. U.S.A. 72: 1314-1316, 1975.

PRAHL, B.: Sutural growth. Investigations on the growth mechanism of the coronal suture and its relation to cranial growth in the rat. Ph.D. Dissentation, University of Nijmegen, Nijmegen, 1968.

PRITCHARD, J.J., SCOTT, J.P. and GIRGIS, R.G.: Structure and development of craniofacial sutures. J. Anat. 90: 73-86, 1956.

PROCKOP, D.J., KIVIRIKKO, K.I., TUDERMAN, L. and GUZMAN, N.A.: The biosynthesis of collagen and its disorders (first of two parts). N. Engl. J. Med. 301: 13-23, 1979a.

PROCKOP, D.J., KIVIRIKKO, K.I., TUDERMAN, L. and GUZMAN, N.A.: The biosynthesis of collagen and its disorders (second of two parts). N. Engl. J. Med. 301: 77-85, 1979b.

PROCKOP, D.J. and WILLIAMS, C.J.: Structure of the organic matrix: collagen structure (chemical). In: Biological Mineralization and Demineralization. Nancollas, G.H. (Ed.), Springer-Verlag, Berlin, pp. 179-198, 1982.

RASMUSSEN, H.: Cell communication, calcium ion and cyclic adenosine monophosphate. Science 170: 404-412, 1970.

REDDI, A.H., GAY, R., GAY, S. and MILLER, E.J.: Transitions in collagen types during matrix-induced cartilage, bone and bone marrow formation. Proc. Natl. Acad. Sci. U.S.A. 74: 5589-5592, 1977.

REITAN, K.: Biomechanical principles and reactions. In: Current Orthodontic Concepts and Techniques. Vol. 1, Graber, T.M. and Swain, B.F. (Eds.), W.B. Saunders and Co., Philadelphia, pp. 111-229, 1975.

RHODES, R.K. and MILLER, E.J.: Physiocochemical characterization and molecular organisation of the collagen A and B chains. Biochemistry 17: 3442-3448, 1978.

ROBERTS, W.E. and JEE, W.S.S.: Cell kinetics of orthodontically stimulated and non-stimulated periodontal ligament in the rat. Arch. Oral Biol. 19: 17-21, 1974.

RODAN, G.A., BOURRET, L.A., HARVEY, A. and MENSI, T.: 3'5'-Cyclic AMP and 3'5'.sk;.of 5cyclic GMP: Mediators of the mechanical effects on bone remodelling. Science 189: 467-469, 1975.

ROLL, F.J., MADRI, J.A., ALBERT, J. and FURTHMAYR, H.: Co-distribution of collagen type IV and AB2 in basement membranes and mesanguim of the kidney. J. Cell Biol. 85:

597-616, 1980.

RYGH, P.: Ultrastructural cellular reactions in pressure zones of rat molar periodontium incident to orthodontic tooth movement. Acta Odont. Scand. 30: 575-593, 1972.

RYGH, P.: Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement. Acta Odont. Scand. 31: 109-122, 1973.

RYGH, P.: Ultrastructural changes in tension zones of rat molar periodontium incident to orthodontic tooth movement. Am. J. Orthodont. 70: 269-281, 1976.

SALTZMAN, L.E., MOSS, J., BERG, R.A., HOM, B. and CRYSTAL, R.G.: Modulation of collagen production by fibroblasts: Effects of chronic exposure to agonists that increase intracellular cyclic AMP. Biochem. J. 204: 25-30, 1982.

SANDSTEDT, C.: Einige Beitrage zur Theorie der Zahnregulierung. Nord. Tandl. Tidsskr, No. 4, 1904. As cited by Schwartz, A.M. Tissue changes incidental to orthodontic tooth movement. Int. J. Orthodont. 18: 331-352, 1932.

SANDSTEDT, C.: Einige Beitrage zur Theorie der Zahnregulierung. Nord. Tandl. Tidsskr, No. 1, 1905a. As cited by Schwartz, A.M. Tissue changes incidental to orthodontic tooth movement. Int. J. Orthodont. 18:331-352, 1932.

SANDSTEDT, C.: Einige Beitrage zur Theorie der Zahnregulierung. Nord. Tandl. Tidsskr, No. 2, 1905b. As cited by Schwartz, A.M. Tissue changes incidental to orthodontic tooth movement. Int. J. Orthodont. 18:331-352, 1932.

SAUK, J.J., GAY, R., MILLER, E.J. and GAY, S.: Immunohistochemical localization of type III collagen in the dentin of patients with osteogenesis imperfecta and hereditary opalescent dentin. J. Oral Path. 9: 210-220, 1980.

SCHWARTZ, A.M.: Tissue changes incidental to orthodontic tooth movement. Int. J. Orthodont. 18: 331-352, 1932.

SCOTT, J.H.: The growth of the human face. Proc. Roy. Soc. Med. 47: 91-100, 1954.

SCOTT, J.H.: Dentofacial Development and Growth. Pergamon Press, Oxford, pp. 89-93, 1967.

SELMAN, A.J. and SARNAT, B.G.: Sutural bone growth of the rabbit snout: a gross and serial roentgenographic study by means of metallic implants. Am. J. Orthodont. 97: 395-408, 1955.

SHACKLEFORD, J.M.: Scanning electron microscopy of the dog

periodontium. J. Periodont. Res. 6: 45-54, 1971.

SICHER, H.: Tooth eruption: The axial movement of continuously growing teeth. J. Dent. Res. 21: 201-210, 1942.

SICHER, H.: The principal fibers of the periodontal membrane. Bur. 55: 2-4, 1954.

SICHER, H.: Changing concepts of the supporting dental structures. Oral Surg. 12: 31-35, 1959.

SICHER, H.: Oral Anatomy. 4th ed., C.V. Mosby Co., St. Louis, Chap. 1, The Skull, pp. 71-75, 1965.

SILBERT, J.E.: Ground substance. In: Textbook of Oral Biology. Shaw, J.H., Sweeney, E.A., Cappuccino, C.C. and Meller, S.M. (Eds.), W.B. Saunders, Philadelphia, pp. 453-469, 1978.

SKILLEN, W.G. and REITAN, K.: Tissue changes following rotation of teeth in the dog. Angle Orthodont. 10: 140-147, 1940.

SKOUGAARD, M.R., LEVY, B.M. and SIMPSON, J.: Collagen metabolism in skin and periodontal membrane of the marmoset. Scand. J. Dent. Res. 78: 256-262, 1970.

SODEK, J.: A new approach to assessing collagen assay by using a micro-assay. A highly efficient and rapid turnover of collagen in rat periodontal tissues. Biochem. J. 160: 243-246, 1976.

SODEK, J.: A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. Arch. Oral Biol. 22: 655-665, 1977.

SODEK, J., BRUNETTE, D.M., FENG, J., HEERSCHE, J.N.M., LIMEBACK, H.F., MELCHER, A.H. and NG, B.: Collagen synthesis is a major component of protein synthesis in the periodontal ligament. Arch. Oral Biol. 22: 647-653, 1977.

SODEK, J., FENG, J., YEN, E.H.K. and MELCHER, A.H.: Effect of ascorbic acid on protein synthesis and collagen hydroxylation in continuous flow organ cultures of adult mouse periodontal tissues. Calcif. Tissue Int. 34: 408-415, 1982.

SODEK, J. and LIMEBACK, H.F.: Comparison of the rates of synthesis, conversion and maturation of type I and type III collagens in rat periodontal tissues. J. Biol. Chem. 254: 10496-10502, 1979.

SOMJEN, D., BINDERMANN, I., BERGER, E. and HANELL, A.: Bone remodelling induced by physical stress in prostaglandin E2 mediated. Biochem. Biophys. Acta 627: 91-100, 1980.

STALLARD, R.E.: The utilization of ^{3}H-proline by the connective tissue elements of the periodontium. Periodontics 1: 185-188, 1963.

STEEL, R.G.D. and JORRIE, J.H.: Principles and Procedures of Statistics. McGraw-Hill, New York, Chap. 8 Analysis of Variance II: Multiway Classification, pp. 156-159, 1960.

STEINMANN, B.U., MARTIN, G.R., BAUM, B.I. and CRYSTAL, R.G.: Synthesis and degradation of collagen by skin fibroblasts from controls and from patients with osteogenesis imperfecta. FEBS Lett. 101: 269-272, 1979.

STEINMANN, B.U. and REDDI, A.H.: Changes in synthesis of type I and III collagen during matrix induced endochondral bone differentiation in rat. Biochem. J. 186: 919-924, 1980.

STERN, B., GLIMCHER, M.J. and GOLDHABER, P.: The effect of various oxygen tensions on the synthesis and degradation of bone collagen in tissue culture. Proc. Soc. Exp. Biol. Med. 127: 869-872, 1966.

STOREY, E.: Growth and remodelling of bone and bones. Am. J. Orthodont. 62: 142-165, 1972.

STOREY, E.: The nature of tooth movement. Am. J. Orthodont. 63: 292-314, 1973.

SYKES, B.C., PUDDLE, B., FRANCIS, M. and SMITH, R.: The estimation of two collagens from human dermis by interrupted gel electrophoresis. Biochem. Biophys. Res. Commun. 72: 1472-1480, 1976.

SZABO, G.: Studies on the cultivation of teeth in vitro. J. Anat. 88: 31-44, 1954.

TANZER, M.L.: Cross-linking of collagen: endogenous aldehydes in collagen react in several ways to form a variety of unique covalent cross-links. Science 180: 561-566, 1973.

TAYLER, B.H., GIANELLY, A.A. and RUBEN, M.P.: Visualization of cellular dynamics associated with orthodontic tooth movement. Am. J. Orthodont. 54: 515-520, 1968.

TEN CATE, A.R.: Morphological studies of fibrocytes in connective tissue undergoing rapid remodelling. J. Anat. 112: 401-414, 1972.

TEN CATE, A.R. and DEPORTER, D.A.: The role of the fibroblast in collagen turnover in the functioning periodontal ligament of the mouse. Arch. Oral Biol. 19: 339-340, 1974.

TEN CATE, A.R. and DEPORTER, D.A.: The degradative role of the

fibroblast in the remodelling and turnover of collagen in soft connective tissue. Anat. Rec. 182: 1-14, 1975.

TEN CATE, A.R., DEPORTER, D.A. and FREEMAN, E.: The role of fibroblasts in the remodelling of periodontal ligament during physiologic tooth movement. Am. J. Orthodont. 69: 155-168, 1976.

TEN CATE, A.R., FREEMAN, E. and DICKINSON, J.B.: Sutural development: structure and its response to rapid expansion. Am. J. Orthodont. 71: 622-636, 1977.

TEN CATE, A.R. and SYRBU, S.: A relationship between alkaline phosphatase activity and the phagocytosis and degradation of collagen by the fibroblast. J. Anat. 117: 351-359, 1974.

THESLEFF, I.: Differentiation of odontogenic tissues in organ culture. Scand. J. Dent. Res. 84: 353-356, 1976.

TONNA, E.A.: Skeletal cell aging and its effects on the osteogenetic potential. Clin. Orthop. 40: 57-81, 1965.

TOOLE, B. and LOWTHER, D.: Dermatin sulfate-protein: Isolation from and interaction with collagen. Arch. Biochem. Biophys. 128: 567-577, 1968.

TROTT, J.R.: The development of the periodontal attachment in the rat. Acta Anat. 51: 313-328, 1962.

TROWELL, O.A.: A modified technique for organ culture in vitro. Exp. Cell Res. 6: 246-248, 1954.

TROWELL, O.A.: The culture of mature organs in a synthetic medium. Exp. Cell Res. 16: 118-147, 1959.

TURNBULL, R.S. and MELCHER, A.H.: A method for maintaining large explants of mature mixed tissue in organ culture: Molar tooth and supporting structures from the mouse. In Vitro 10: 6-11, 1974.

WAERHAUG, J.: Effect of C-avitaminosis on the supporting structures of the teeth. J. Periodontol. 29: 87-97, 1958.

WALDO, C.M. and ROTHBLATT, J.M.: Histologic response to tooth movement in the laboratory rat. J. Dent. Res. 33: 481-486, 1954.

WATANABE, M., LASKIN, D.M. and BRODIE, A.G.: The effect of autotransplantation on growth of the zygomatico-maxillary suture. Am. J. Anat. 100: 319-335, 1957.

WAYMOUTH, C.: Rapid proliferation of sublines NCTC clone 929 (strain L) mouse cells in a simple chemically defined medium.

J. Nat. Cancer Inst. 22: 1003-1015, 1959.

WEINMANN, J.P.: The adaptation of the periodontal membrane to physiologic and pathologic changes. Oral Surg. 8: 977-981, 1955.

WEINMANN, J.P. and SICHER, H.: Bone and Bones. C.V. Mosby Co., St. Louis, 1947.

WEINMANN, J.P. and SICHER, H.: Bone and Bones. 2nd ed., C.V. Mosby Co., St. Louis, Chap. II Bones, pp. 88-91, 1955.

WEISS, J.B., SHUTTLEWORTH, C.A., BROWN, R., SEDOWFIA, K., BAILDAM, A. and HUNTER, J.A.: Occurrence of type III collagen in inflamed synovial membranes: A comparison between non-rheumatoid, rheumatoid and normal synovial collagens. Biochem. Biophys. Res. Commun. 65: 907-912, 1975.

WEISS, R., STAHL, S.S. and TONNA, E.A.: Functional demands on the cell proliferative activity of the rat periodontium studied autoradiographically. J. Dent. Res. 47: 1153-1157, 1968.

YAMASAKI, K., MUIRA, F. and SUDA, T.: Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. J. Dent. Res. 59: 1635-1642, 1980.

YEE, J.A.: Response of periodontal ligament cells to orthodontic force. Ultrastructural identification of proliferating fibroblasts. Anat. Rec. 194: 603-614, 1979.

YEE, J.A., KIMMEL, D.B. and JEE, W.S.S.: Periodontal ligament cell kinetics following orthodontic tooth movement. Cell Tissue Kinet. 9: 293-302, 1976.

YEN, E.H.K.: Organ culture of adult mouse molar periodontium. Effect of oxygen tension on protein synthesis. Ph.D. Thesis, University of Toronto, Toronto, 1978.

YEN, E.H.K. and CHIANG, S.K.T.: The effect of maturation on the protein synthetic and bone deposition activity in the inter-parietal suture of male white mice: A radioautographic study. Arch. Oral Biol., (in press).

YEN, E.H.K., DUNCAN, G.W. and SUGA, D.M.: Biological response to orthodontic and orthopaedic forces in vitro: Collagen synthesis. In: Malocclusion and the Periodontium. McNamara, J.A. Jr. and Ribbens, K.A. (Eds.), Centre for Growth and Development, The University of Michigan, Ann Arbor, 1984.

YEN, E.H.K. and MELCHER, A.H.: A continuous flow culture system for organ culture of large explants of adult tissue. Effect of oxygen tension on mouse molar periodontium In Vitro 14:

811-818, 1978.

YEN, E.H.K. and SUGA, D.: Collagen and non-collagenous protein synthesis in adult mouse cranial suture in vitro. J. Dent. Res. 60 (Special Issue A): (Abstract 1078), 1981.

YEN, E.H.K., SUGA, D. and CHIANG, S.: Identification of collagen types synthesized in interparietal suture during orthopaedic stress. J. Dent. Res. 59 (Special Issue B): (Abstract 60), 1980.

YOUNG, R.W.: The influence of cranial contents on postnatal growth of the skull in the rat. Am. J. Anat. 105: 383-415, 1959.

YOUNG, R.W.: Autoradiographic studies on postnatal growth of the skull in young rats injected with tritiated glycine. Anat. Rec. 143: 1-14, 1962.

ZACCHARISON, B.U.: Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. Am. J. Orthodont. 69: 285-300, 1976.

ZAKI, A.E. and VAN HUYSEN, G.: Histology of the periodontium following tooth movement. J. Dent. Res. 42: 1373-1379, 1963.

ZENGO, A.N., BASSETT, C.A.L., PAWLUK, R.J. and PROUNTZOS, G.: In vivo bioelectric potentials in the dentoalveolar complex. Am. J. Orthodont. 66: 130-139, 1974.

ZENGO, A.N., BASSETT, C.A.L., PROUNTZOS, G., PAWLUK, R.J. and DILLA, A.: In vivo effects of direct current in the mandible. J. Dent. Res. 55: 383-390, 1976.

ZENGO, A.N., PAWLUK, R.J. and BASSETT, C.A.L.: Stress-induced bioelectric potentials in the dentoalveolar complex. Am. J. Orthodont. 64: 17-27, 1973.

ZWARYCH, P.D. and QUIGLEY, M.B.: The intermediate plexus of the periodontal ligament: history and further observations. J. Dent. Res. 44: 383-391, 1965.

ZIMRING, J. and ISAACSON, R.: Forces produced by rapid maxillary expansion. III. Forces present during retention. Angle Orthodont. 35: 178-186, 1965.

APPENDIX

SUPPLEMENTARY TABLES

TABLE VI-2

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 6 HOURS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 0.67	35 gm 0.50	25 gm 0.50	15 gm 0.33	5 gm 0.00
50 gm 0.67		**	**	**	**
35 gm 0.50	**		NS	**	**
25 gm 0.50	**	NS		**	**
15 gm 0.33	**	**	**		**
5 gm 0.00	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI- 3

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 1 DAY OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 1.00	35 gm 0.67	25 gm 0.67	15 gm 0.33	5 gm 0.00
50 gm 1.00		**	**	**	**
35 gm 0.67	**		NS	**	**
25 gm 0.67	**	NS		**	**
15 gm 0.33	**	**	**		**
5 gm 0.00	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-4

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 3 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 1.22	35 gm 1.33	25 gm 1.00	15 gm 0.67	5 gm 0.44
50 gm 1.22		*	**	**	**
35 gm 1.33	*		**	**	**
25 gm 1.00	**	**		**	**
15 gm 0.67	**	**	**		**
5 gm 0.44	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-5

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 5 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 1.56	35 gm 1.33	25 gm 1.11	15 gm 1.00	5 gm 0.56
50 gm 1.56		*	**	**	**
35 gm 1.33	*		**	**	**
25 gm 1.11	**	**		NS	**
15 gm 1.00	**	**	NS		**
5 gm 0.56	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-6

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 7 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 1.67	35 gm 1.50	25 gm 1.33	15 gm 1.33	5 gm 0.50
50 gm 1.67		NS	**	**	**
35 gm 1.50	NS		NS	NS	**
25 gm 1.33	**	NS		NS	**
15 gm 1.33	**	NS	NS		**
5 gm 0.50	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-7

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 10 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 2.00	35 gm 1.56	25 gm 1.50	15 gm 1.33	5 gm 0.78
50 gm 2.00		**	**	**	**
35 gm 1.56	**		NS	**	**
25 gm 1.50	**	NS		*	**
15 gm 1.33	**	**	*		**
5 gm 0.78	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-8

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 14 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 2.00	35 gm 1.84	25 gm 1.78	15 gm 1.61	5 gm 1.33
50 gm 2.00		NS	**	**	**
35 gm 1.84	NS		NS	NS	**
25 gm 1.78	**	NS		NS	**
15 gm 1.61	**	NS	NS		**
5 gm 1.33	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-9

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 21 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 2.00	35 gm 2.00	25 gm 1.89	15 gm 1.89	5 gm 1.50
50 gm 2.00		NS	NS	NS	**
35 gm 2.00	NS		NS	NS	**
25 gm 1.89	NS	NS		NS	**
15 gm 1.89	NS	NS	NS		**
5 gm 1.50	**	**	**	**	

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-10

MULTIPLE t-TEST FOR SUTURE EXPANSION AFTER 28 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	50 gm 2.00	35 gm 2.00	25 gm 2.00	15 gm 2.00	5 gm 2.00
50 gm 2.00	X	NS	NS	NS	NS
35 gm 2.00	NS	X	NS	NS	NS
25 gm 2.00	NS	NS	X	NS	NS
15 gm 2.00	NS	NS	NS	X	NS
5 gm 2.00	NS	NS	NS	NS	X

(†) Retransformed data. Numbers indicate means of expansion (mm).
Pooled standard error = 1.01 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-12

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE CONTROL MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 3.72	1 day 3.82	3 days 3.27	5 days 3.69	7 days 3.94	10 days 3.00	14 days 0.69	21 days 0.00	28 days 0.00
6 hours 3.72		NS	NS	NS	NS	NS	**	**	**
1 day 3.82	NS		NS	NS	NS	NS	**	**	**
3 days 3.27	NS	NS		NS	NS	NS	**	**	**
5 days 3.69	NS	NS	NS		NS	NS	**	**	**
7 days 3.94	NS	NS	NS	NS		NS	**	**	**
10 days 3.00	NS	NS	NS	NS	NS		**	**	**
14 days 0.69	**	**	**	**	**	**		NS	NS
21 days 0.00	**	**	**	**	**	**	NS		NS
28 days 0.00	**	**	**	**	**	**	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI- 13

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE SHAM-OPERATED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 2.29	1 day 11.29	3 days 14.10	5 days 12.92	7 days 12.53	10 days 8.39	14 days 4.24	21 days 3.82	28 days 3.61
6 hours 2.29		**	**	**	**	**	*	NS	NS
1 day 11.29	**		**	NS	NS	**	**	**	**
3 days 14.10	**	**		NS	NS	**	**	**	**
5 days 12.92	**	NS	NS		NS	**	**	**	**
7 days 12.53	**	NS	NS	NS		**	**	**	**
10 days 8.39	**	**	**	**	**		**	**	**
14 days 4.24	*	**	**	**	**	**		NS	NS
21 days 3.82	NS	**	**	**	**	**	NS		NS
28 days 3.61	NS	**	**	**	**	**	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-14

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE 50 GM TENSILE STRESSED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 1.08	1 day 14.82	3 days 19.38	5 days 9.05	7 days 8.77	10 days 7.62	14 days 8.10	21 days 7.19	28 days 7.58
6 hours 1.08		**	**	**	**	**	**	**	**
1 day 14.82	**		**	**	**	**	**	**	**
3 days 19.38	**	**		**	**	**	**	**	**
5 days 9.05	**	**	**		NS	NS	NS	*	NS
7 days 8.77	**	**	**	NS		NS	NS	NS	NS
10 days 7.62	**	**	**	NS	NS		NS	NS	NS
14 days 8.10	**	**	**	NS	NS	NS		NS	NS
21 days 7.19	**	**	**	*	NS	NS	NS		NS
28 days 7.58	**	**	**	NS	NS	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-15

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE 35 GM TENSILE STRESSED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 2.49	1 day 14.96	3 days 18.05	5 days 14.07	7 days 10.53	10 days 6.10	14 days 4.57	21 days 4.93	28 days 4.72
6 hours 2.49		**	**	**	**	**	**	**	**
1 day 14.96	**		**	NS	**	**	**	**	**
3 days 18.05	**	**		**	**	**	**	**	**
5 days 14.07	**	NS	**		**	**	**	**	**
7 days 10.53	**	**	**	**		**	**	**	**
10 days 6.10	**	**	**	**	**		NS	NS	NS
14 days 4.57	**	**	**	**	**	NS		NS	NS
21 days 4.93	**	**	**	**	**	NS	NS		NS
28 days 4.72	**	**	**	**	**	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-16

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE 25 GM TENSILE STRESSED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 3.08	1 day 15.06	3 days 19.33	5 days 16.38	7 days 6.21	10 days 4.98	14 days 5.30	21 days 3.55	28 days 3.28
6 hours 3.08		**	**	**	**	*	**	NS	NS
1 day 15.06	**		**	NS	**	**	**	**	**
3 days 19.33	**	**		**	**	**	**	**	**
5 days 16.38	**	NS	**		**	**	**	**	**
7 days 6.21	**	**	**	**		NS	NS	**	**
10 days 4.98	*	**	**	**	NS		NS	NS	*
14 days 5.30	**	**	**	**	NS	NS		*	**
21 days 3.55	NS	**	**	**	**	NS	*		NS
28 days 3.28	NS	**	**	**	**	*	**	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-17

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE 15 GM TENSILE STRESSED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 5.42	1 day 16.85	3 days 13.85	5 days 13.34	7 days 7.23	10 days 4.97	14 days 3.21	21 days 3.26	28 days 3.65
6 hours 5.42		**	**	**	*	NS	**	**	**
1 day 16.85	**		*	**	**	**	**	**	**
3 days 13.85	**	*		NS	**	**	**	**	**
5 days 13.34	**	**	NS		**	**	**	**	**
7 days 7.23	*	**	**	**		**	**	**	**
10 days 4.97	NS	**	**	**	**		*	*	NS
14 days 3.21	**	**	**	**	**	*		NS	NS
21 days 3.26	**	**	**	**	**	*	NS		NS
28 days 3.65	*	**	**	**	**	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-18

ANALYSIS OF VARIANCE OF MEAN VALUES OF RATIOS OF TYPE III COLLAGEN FOR THE 5 GM TENSILE STRESSED MOUSE INTERPARIETAL SUTURE OVER THE TIME PERIOD STUDIED (†)

	6 hrs 6.11	1 day 13.21	3 days 13.64	5 days 13.24	7 days 7.79	10 days 5.45	14 days 5.14	21 days 3.84	28 days 2.39
6 hours 6.11		**	**	**	NS	NS	NS	**	**
1 day 13.21	**		NS	NS	**	**	**	**	**
3 days 13.64	**	NS		NS	**	**	**	**	**
5 days 13.24	**	NS	NS		**	**	**	**	**
7 days 7.79	NS	**	**	**		**	**	**	**
10 days 5.45	NS	**	**	**	**		NS	NS	**
14 days 5.14	NS	**	**	**	**	NS		NS	**
21 days 3.84	**	**	**	**	**	NS	NS		NS
28 days 2.39	**	**	**	**	**	**	**	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.

* = Significant difference at P = 0.05 confidence level.

** = Significant difference at P = 0.01 confidence level.

TABLE VI-19

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 6 HOURS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.72	Sham-Operated 2.29	50 gm 1.08	35 gm 2.49	25 gm 3.08	15 gm 5.42	5 gm 6.11
Control 3.72		NS	NS	NS	NS	NS	NS
Sham-operated 2.29	NS		NS	NS	NS	NS	*
50 gm 1.08	NS	NS		NS	NS	*	**
35 gm 2.49	NS	NS	NS		NS	NS	NS
25 gm 3.08	NS	NS	NS	NS		NS	NS
15 gm 5.42	NS	NS	*	NS	NS		NS
5 gm 6.11	NS	*	**	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI- 20

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 1 DAY OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.82	Sham-Operated 11.29	50 gm 14.82	35 gm 14.96	25 gm 15.06	15 gm 16.85	5 gm 13.21
Control 3.82		**	**	**	**	**	**
Sham-operated 11.29	**		*	*	*	**	NS
50 gm 14.82	**	*		NS	NS	NS	NS
35 gm 14.96	**	*	NS		NS	NS	NS
25 gm 15.06	**	*	NS	NS		NS	NS
15 gm 16.85	**	**	NS	NS	NS		*
5 gm 13.21	**	NS	NS	NS	NS	*	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-21

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 3 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.27	Sham-Operated 14.10	50 gm 19.38	35 gm 18.05	25 gm 19.33	15 gm 13.85	5 gm 13.64
Control 3.27		**	**	**	**	**	**
Sham-operated 14.10	**		**	*	**	NS	NS
50 gm 19.38	**	**		NS	NS	**	**
35 gm 18.05	**	*	NS		NS	*	*
25 gm 19.33	**	**	NS	NS		**	**
15 gm 13.85	**	NS	**	*	**		NS
5 gm 13.64	**	NS	**	*	**	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-22

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 5 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.69	Sham-Operated 12.92	50 gm 9.05	35 gm 14.07	25 gm 16.38	15 gm 13.34	5 gm 13.24
Control 3.69		**	**	**	**	**	**
Sham-operated 12.92	**		*	NS	NS	NS	NS
50 gm 9.05	**	*		**	**	*	*
35 gm 14.07	**	NS	**		NS	NS	NS
25 gm 16.38	**	NS	**	NS		NS	NS
15 gm 13.34	**	NS	*	NS	NS		NS
5 gm 13.24	**	NS	*	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-23

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 7 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.94	Sham-Operated 12.53	50 gm 8.77	35 gm 10.53	25 gm 6.21	15 gm 7.23	5 gm 7.79
Control 3.94		**	**	*	NS	NS	*
Sham-operated 12.53	**		*	NS	**	**	*
50 gm 8.77	**	*		NS	NS	NS	NS
35 gm 10.53	*	NS	NS		*	NS	NS
25 gm 6.21	NS	**	NS	*		NS	NS
15 gm 7.23	NS	**	NS	NS	NS		NS
5 gm 7.79	*	*	NS	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-24

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 10 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 3.00	Sham-Operated 8.39	50 gm 7.62	35 gm 6.10	25 gm 4.98	15 gm 4.97	5 gm 5.45
Control 3.00		**	**	NS	NS	NS	NS
Sham-operated 8.39	**		NS	NS	NS	NS	NS
50 gm 7.62	**	NS		NS	NS	NS	NS
35 gm 6.10	NS	NS	NS		NS	NS	NS
25 gm 4.98	NS	NS	NS	NS		NS	NS
15 gm 4.97	NS	NS	NS	NS	NS		NS
5 gm 5.45	NS	NS	NS	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-25

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 14 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 0.69	Sham-Operated 4.24	50 gm 8.10	35 gm 4.57	25 gm 5.30	15 gm 3.21	5 gm 5.14
Control 0.69		NS	**	NS	*	NS	*
Sham-operated 4.24	NS		*	NS	NS	NS	NS
50 gm 8.10	**	*		NS	NS	*	NS
35 gm 4.57	NS	NS	NS		NS	NS	NS
25 gm 5.30	*	NS	NS	NS		NS	NS
15 gm 3.21	NS	NS	*	NS	NS		NS
5 gm 5.14	*	NS	NS	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-26

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 21 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 0.00	Sham-Operated 3.82	50 gm 7.19	35 gm 4.93	25 gm 3.55	15 gm 3.26	5 gm 3.84
Control 0.00		*	**	*	NS	NS	NS
Sham-operated 3.82	*		NS	NS	NS	NS	NS
50 gm 7.19	**	NS		NS	NS	*	NS
35 gm 4.93	*	NS	NS		NS	NS	NS
25 gm 3.55	NS	NS	NS	NS		NS	NS
15 gm 3.26	NS	NS	*	NS	NS		NS
5 gm 3.84	NS	NS	NS	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.

TABLE VI-27

MULTIPLE t-TEST FOR MEAN VALUES OF RATIOS OF TYPE III COLLAGEN AFTER 28 DAYS OF TENSILE STRESS AT DIFFERENT FORCE LEVELS (†)

	Control 0.00	Sham-Operated 3.61	50 gm 7.58	35 gm 4.72	25 gm 3.28	15 gm 3.65	5 gm 2.39
Control 0.00		NS	**	*	NS	NS	NS
Sham-operated 3.61	NS		*	NS	NS	NS	NS
50 gm 7.58	**	*		NS	*	*	**
35 gm 4.72	*	NS	NS		NS	NS	NS
25 gm 3.28	NS	NS	*	NS		NS	NS
15 gm 3.65	NS	NS	*	NS	NS		NS
5 gm 2.39	NS	NS	**	NS	NS	NS	

(†) Retransformed data. Numbers indicate means of type III/type I + III (%). Pooled standard error = 1.08 and applied to the means multiplicatively.

NS = No significant difference.
* = Significant difference at P = 0.05 confidence level.
** = Significant difference at P = 0.01 confidence level.