# THE TEMPOROMANDIBULAR JOINT OF THE RAT :

# EFFECTS OF OCCLUSAL DISHARMONY AND DISTURBANCES IN COLLAGEN METABOLISM

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# PRECIS

This study was designed to investigate the possible effects of loss of posterior occlusion (mandibular overclosure) on the temporomandibular joint of the rat. The normal anatomy and histology of the temporomandibular joint was studied, together with the muscles of mastication, the teeth and occlusion. The functional aspects have been discussed and compared with several other animals with different functional occlusions (functional occlusions of these animals other than the rat were the subject of a previous study: Lam 1971).

In one series of rats, the maxillary molar teeth were removed to determine the effect of loss of posterior occlusion on the temporomandibular joint.

In another series, a lathyrogenic diet was fed to the rats in order to determine the effects of lathyrism on the temporomandibular joint.

In a final series, a lathyrogenic diet was fed to rats whose maxillary molars had been removed, in order to determine the effects of loss of posterior occlusion on the lathyritic joint.

The following results were obtained:

(1) The lateral pterygoid muscle was found to have two separate insertions, a fact which can be explained on

the basis of functional requirements within the temporo-mandibular joint;

(2) In the lathyritic joint, changes in the mandibular condyle, including the site of insertion of the lateral pterygoid muscle, were noted;

(3) In the lathyritic joint with loss of posterior occlusion temporal bone resorption and posterior condylar surface irregularity were noted in addition to changes already noted in lathyrism alone;

(4) In both (2) and (3) there was hyperplasia of connective tissue at the insertion of the lateral pterygoid forming an "exostosis".

The effects obtained in the lathyritic joints were considered to be the result of stress on abnormal collagen.

The results of this study in some respects confirm the findings of other investigators, but in some respects differ from them.

## DECLARATION

This thesis is submitted in part fulfilment of the requirements for the Degree of Master of Dental Surgery in the University of Adelaide. Candidature for the Degree was satisfied by a Qualifying Examination in 1971.

This thesis contains no material which, except where due mention is made, has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

K.P. Lam

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# INTRODUCTION

In recent years it has become recognised by all workers in the field of Occlusion that the Masticatory System is not merely a collection of anatomical components comprised of the teeth, the masticatory muscles, the nervous system supplying these structures, and the temporomandibular joint, but is a co-ordinated system of all these components functioning as a unit. This functional concept implies harmony among the components and dynamic equilibrium with one another. Thus if one component is not functioning properly, the others will be in hyper- or hypo-function in an attempt to maintain the original equilibrium --- this being the basis of functional adaptation. If functional adaptation does not occur, or if it is inadequate, pathology is the ultimate result.

The first Section of this thesis is devoted to an examination of the units of the Masticatory System of the Rat, and the concept of Functional Occlusion is established by demonstrating the intimate relationship between its components. The structures of the temporomandibular joint will be examined in detail in Section I, as they will be the main subject of experimental pathology in Sections II, III, and IV. The findings of this Section will establish a working baseline for the examination of temporomandibular joint structures which have been subjected to controlled experimental procedures. Also, the controversy on the lateral pterygoid muscle concerning its anatomy and function (Christensen 1969, Lam 1971) will be further investigated and discussed.

Section II deals with an investigation of possible effects of loss of posterior occlusion on the structures of the temporomandibular joint. That this loss of posterior teeth could cause symptoms in the region of the face and ear has been known for many years. Costen (1934, 1936, 1939, 1944, 1956) described the association of mandibular overclosure with distal displacement of the mandibular condyle in the joint, causing peri-auricular pain, impaired hearing, tinnitus, glossodynia, and pain in the temporal region and in the face. Lammie, Storer and Osborne (1956) also pointed out how loss of posterior teeth could lead to distal malpositioning of the mandibular condyle by the action of an inclined plane between the anterior teeth. Steinhardt (1932) and Shore (1959) also discussed the important relationship between occlusion and the temporomandibular joint. The true cause of all these conditions, however, has remained obscure. The original explanation by Costen has now been replaced by the concept of muscular pain in various areas of the head and jaws caused by malocclusion (including loss of occlusion) which disturbs the equilibrium between the occlusion and muscle function (Schwartz 1959, Sicher 1955, Perry 1957, Travell 1960). The articular meniscus could also be involved in many cases of temporomandibular joint dysfunction, as is the case where there is crepitus resulting from disharmonious movements between the meniscus and the two articular surfaces (Christensen 1969, Ireland 1951, Berry & Hofmann 1956). In view of its possession of a nerve supply (Griffin et al. 1965, Dixon 1962, Bernich 1962, Christensen 1969), pathological involvement of the meniscus in malocclusion and loss of occlusion could make a real contribution towards facial pain. One of the major questions in connection with this subject is: Do

any pathological changes occur within the joint as a result of loss of posterior occlusion and mandibular overclosure? In order to obtain some insight into this problem some workers have used experimental animals and studied their joints histologically after certain time intervals following extraction. Some workers have examined the joints after raising the occlusion (Gianelly, Ruben & Risinger 1970, Applebaum & Levy 1954) but not after decreasing occlusion. Some workers have examined the joints following extraction of posterior teeth (Pietrokovski 1970) and traumatic occlusion (Ruben & Mafla 1971), but on monkeys only. Other workers have extracted teeth from rats (Cimasoni 1963, Furstman 1965) but such work to date has been scanty and the results were controversial. Thus, Cimasoni reported the following histopathological changes in the rat's temporomandibular joints: discrete calcifications in the temporal fossa, necrosis of cartilage in the fossa, isolated alterations in the structure of the meniscus, and the formation of a vascular pannus in the upper joint space. However, Furstman (1965) in his series of Rat joints reported none of the above findings except some disorientation of fibrous tissues in the joint; on the other hand he found osteosclerosis in squamosal and condylar bone (not reported by Cimasoni 1963), a thickening of the meniscus and a decrease in width of the cartilagenous cap of the condyle. These latter findings are questionable; as he used coronal sections of the joint only, the thickness of the meniscus as it appeared on the sections depended entirely on where the coronal section was cut; if it was sectioned through the Anterior and Posterior Thick Zones it would be thick, and if it was through the Intermediate Thin Zone it would be thin (see p.1.25 to 1.27). Unless the area of sectioning is specified, the thickness and thinness of the meniscus in

a coronal section is meaningless. As for the decrease of cartilagenous cap width, its validity is seen to be untenable after a critical examination (as discussed in detail in Chapter 7). In Section II of this thesis I have therefore set out to examine the joints both sagittally and coronally, at post-operative intervals of from 2 days to 3 months. A similar study which extended for 3 months using joints affected by lathyrism will be the subject of Section IV.

Weakening of joint structures in order to accentuate structural changes following extraction of posterior teeth can be achieved by inducing lathyrism in rats (The theory and methods used to produce lathyrism are discussed in Chapter 9 and Appendix IV). However, before any conclusions can be drawn, the effects of lathyrism alone on the joint structures must be determined first; this is the purpose of Section III of this study. Apart from forming a controlling baseline for Section IV, this Section should be in itself an interesting study showing any histological alterations in the tissues of the temporomandibular joint of rats in which there is progressively weaker collagen in the body generally.

# SECTION I

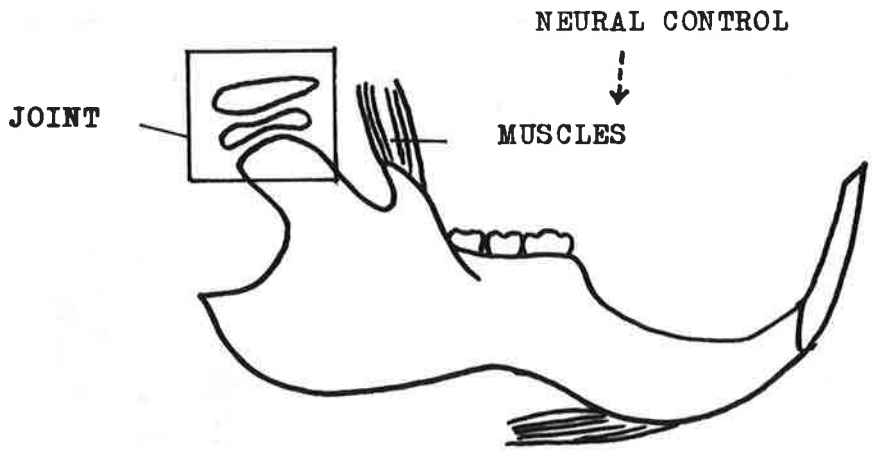
## NORMAL STRUCTURE AND FUNCTION OF THE RAT TEMPOROMANDIBULAR JOINT

## COMPONENTS OF THE MASTICATORY SYSTEM OF THE RAT

The Temporomandibular Joint of the Rat consists of (1) the temporal articular surface, (2) the mandibular condyle, and (3) the articular meniscus. These structures are the subject of study in relation to normal function and experimental pathology, and will therefore be discussed in detail.

The functionally related structures of the Joint are (1) the masticatory muscles: masseter, temporalis, medial pterygoid, lateral pterygoid, and the anterior belly of the digastric, (2) the teeth and occlusion, and (3) the nervous system controlling and co-ordinating these structures.

Fig. 1.1 THE TEMPOROMANDIBULAR JOINT and the Masticatory System:



# CHAPTER 1

## THE TEMPOROMANDIBULAR JOINT OF THE RAT

### (A) GROSS ANATOMY:

### TEMPORAL ARTICULAR SITE

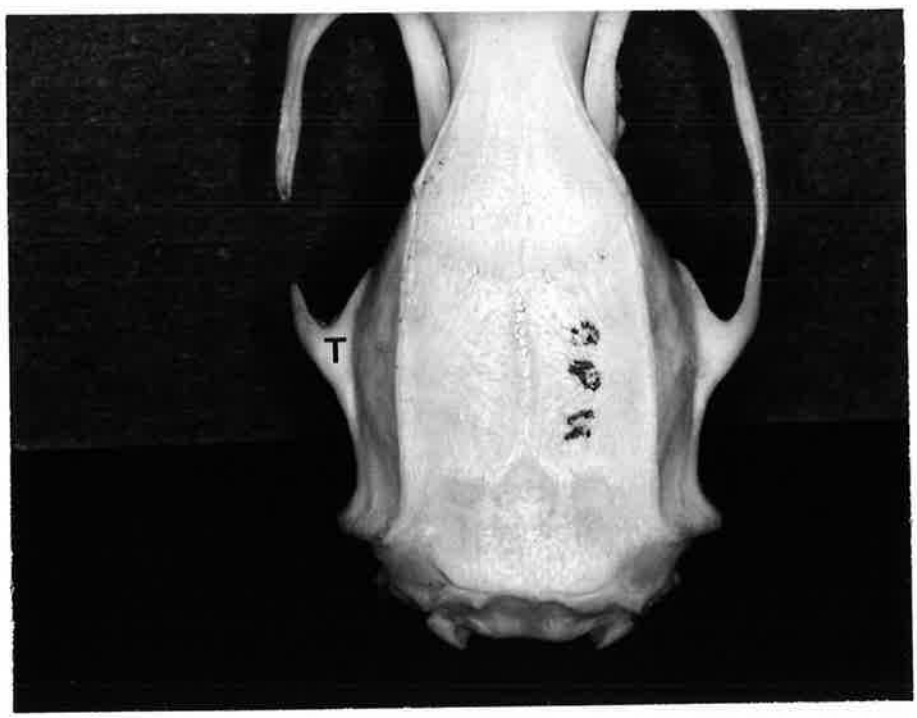
The temporal articular site is a curved flange extending from the temporal bone as the zygomatic process. Not all of this process is involved in the Joint --- only its most posterior part. Anteriorly, the zygomatic process of the temporal bone is smoothly continuous with the posterior part of the temporal process of the zygomatic bone, forming jointly the Zygomatic Arch (Fig. 1.2).

Fig. 1.2 Lateral view of Rat Skull (Left side)
Z = Zygomatic process
T = Temporal articular site

Viewed laterally, the temporal articular site begins as a ridge heaped up from the lateral aspect of the temporal bone, extending as far posteriorly as the auditory canal, and anteriorly as the posterior wall of the orbit. This is continued laterally in the form of a flange which roofs the temporomandibular joint. The flange gradually tapers to form the zygomatic arch at the same time as it continues infero-anteriorly to leave the joint.

Viewed from above, the medial part of the temporal site which is described as the "ridge" above is seen to terminate anteriorly at the posterior wall of the orbit as an abrupt stoppage (Fig. 1.3) but only to merge slowly into the rest of the temporal bone posteriorly.

Fig. 1.3 Superior view of skull
T = Temporal articular site



Viewed from posteriorly, it can be seen that the site is in the form of a "gutter", having a medial wall, an arched roof, and a lateral flange, but there is no anterior or posterior flange. The mandibular condyle is therefore able to move freely antero-posteriorly within the joint, but only to an extremely limited extent in the medio-lateral direction (Fig. 1.4 and 1.5).

Fig. 1.4 Postero-inferior view
G = "Gutter"

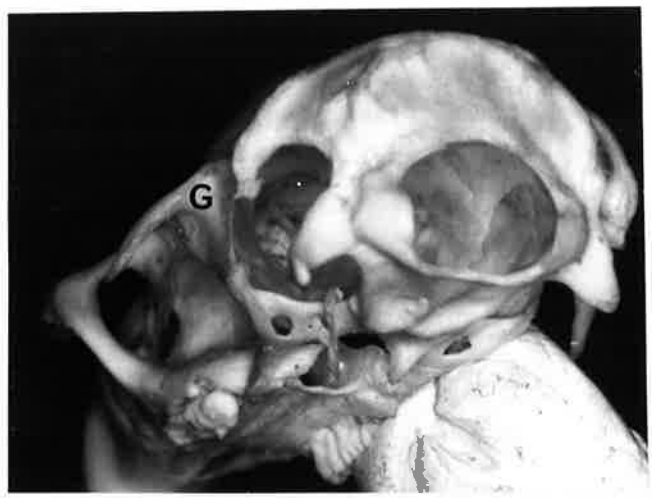
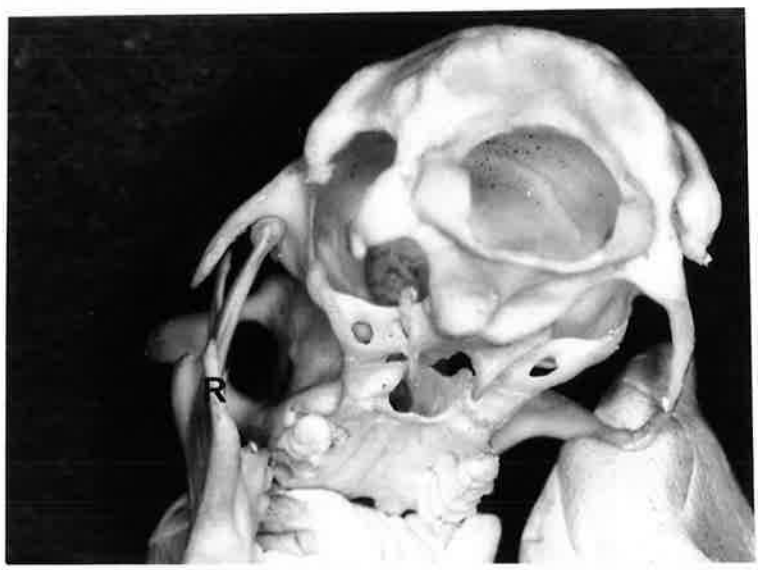



Fig. 1.5 Condyle within the Joint
R = Ramus of mandible



## MANDIBULAR CONDYLE

The mandibular condyle is continuous with the condylar process of the mandible. The condylar process is in the form of a sheet drawn out from the ascending ramus, broad anteroposteriorly but thin mediolaterally. The condyle is the superior part of this sheet, and is the articular part of the condylar process involved within the joint. The condyle is thicker than the rest of the process, and is convex throughout.

Viewed from the lateral aspect, the condyle is a convex structure pointing postero-superiorly. Its antero-posterior dimension is in the form of an arc. A sharp ridge marks the inferior limit of the condyle, and this ridge ends as an inverted lip at the most lateral part of the condyle as the "Lateral lip" (Fig. 1.6 and 1.7).

Fig. 1.6 Lateral view of Condyle (Left side)
CP = Condylar Process

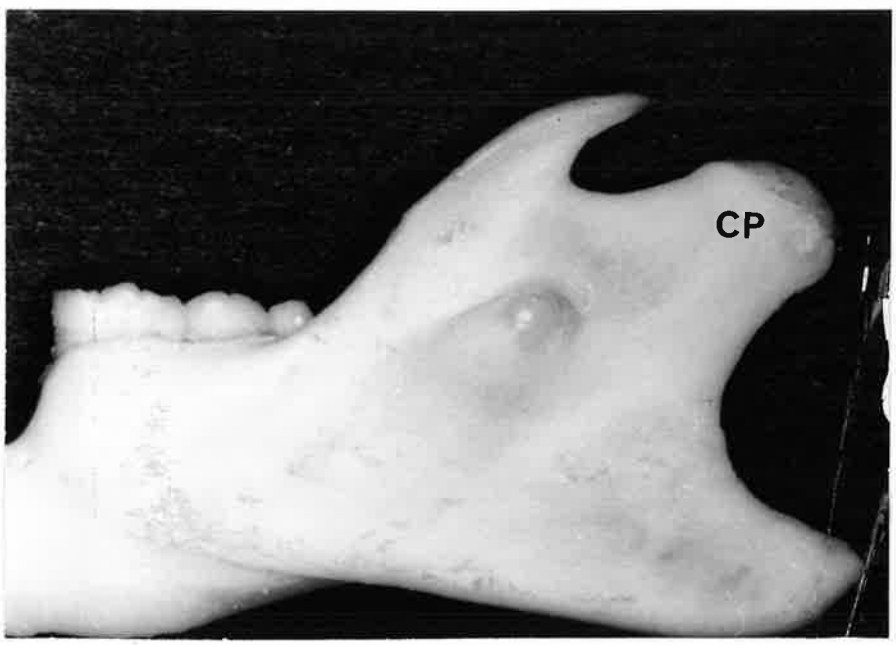
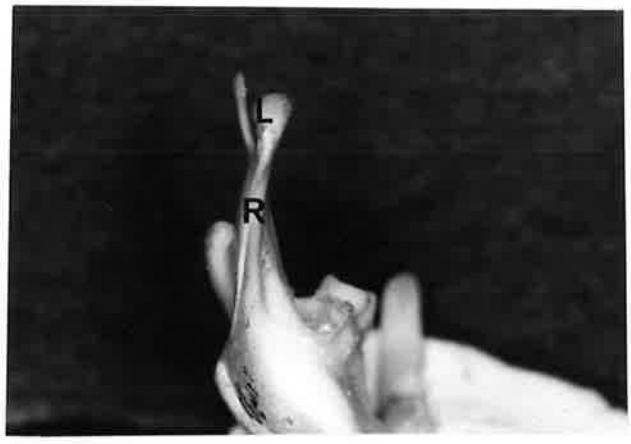


Fig. 1.7 Posterior view of Condyle (Left side)

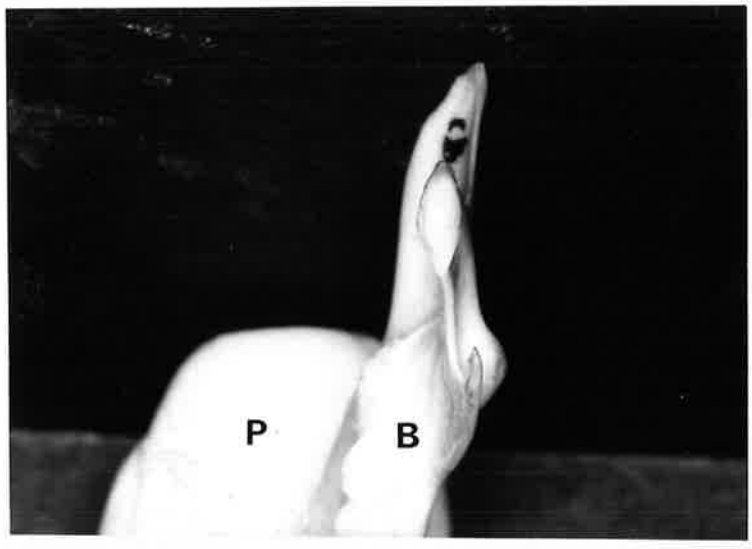
R = Ramus
L = Lateral lip




Viewed from above, the condyle is fusiform, its antero-posterior dimension being three times the dimension mediolaterally. It terminates anteriorly at a definite point which is structurally continuous with the rest of the ramus. The direction of the condyle is mainly antero-posterior but with a slight medial inclination (Fig. 1.8).

Fig. 1.8 Superior aspect of Condyle

B = Body of mandible
P = Plasticine used during photography



Viewed from behind, the condyle is thicker superiorly than inferiorly. The lateral and medial lips are again seen, but the lateral lip is seen to be lower than the medial one. The condyle has a supero-medial inclination (Fig. 1.7).

While the condyle is thicker than the rest of the process, it is really only about 1.5mm wide mediolaterally. Consequently the temporomandibular joint is limited to about 1.5mm in its total width.

## ARTICULAR MENISCUS

Owing to its small size, the articular meniscus is better described under the section of Histology as presented below (p.1.25), where it has been subjected to serial sectioning and its shape, structure, and attachments can be examined microscopically.

## TEMPORAL SITE AND CONDYLAR ARTICULATION

Articulated together, it can be seen that the condyle can rotate freely on the temporal site in the sagittal plane. This is what occurs during opening and closing of the mouth. The condyle can also slide antero-posteriorly, which is important for antero-posterior grinding by the molar teeth when chewing food.

With the temporal "gutter" housing the condyle snugly between its medial and lateral walls, the condyle

cannot move more than a negligible amount medio-laterally. This medial and lateral limitation serves as a definite guide in the antero-posterior movements of the condyle within the temporomandibular joint space.

Fig. 1.9 Lateral view of Articulated Joint
(Left side)

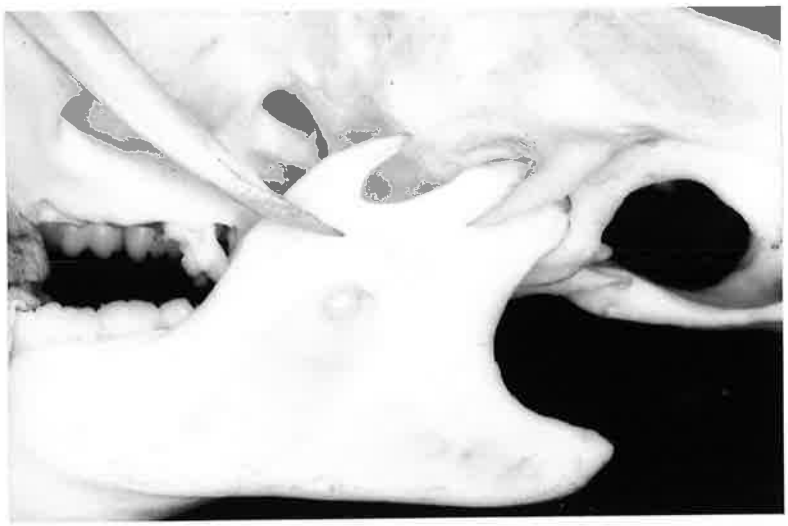
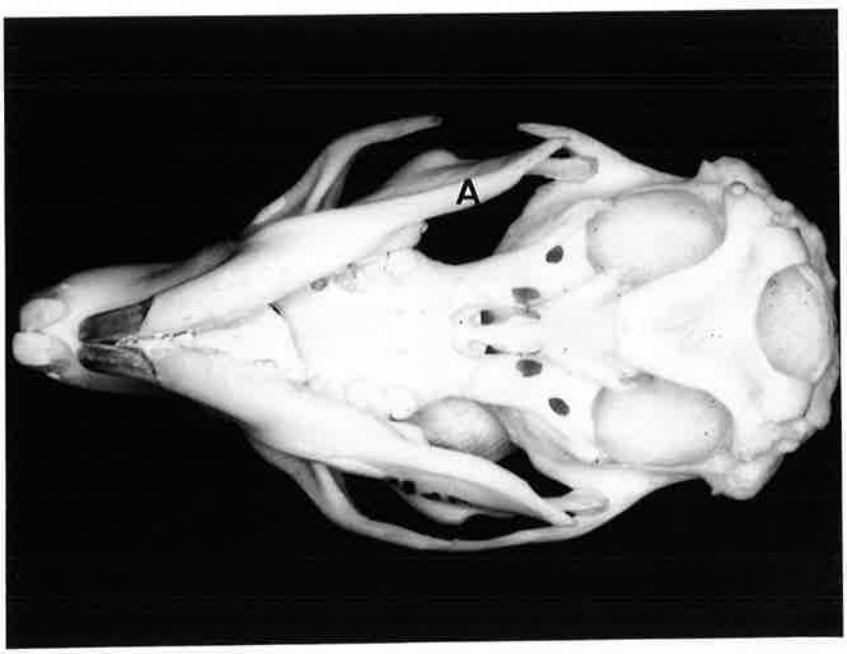


Fig. 1.10 Inferior view of Articulated Joint
A = Angle of mandible



## (B) HISTOLOGY:

A study of the Joint Structures includes examination of the distribution of (1) fibrous connective tissues, (2) cartilage, and (3) bone. A brief description of the histology and biochemistry of these tissues is given here to serve as a background to an understanding of the histology and histophysiology described later.

### CONNECTIVE TISSUES : FIBROUS CONNECTIVE TISSUE

The connective tissues are widely distributed in the body. They are derived from mesenchyme, and consist of cells and intercellular matrix (extracellular substances). Their name is illustrative of their functions, which are: (1) to connect and bind tissues together, e.g. the fasciae among groups of muscles; and (2) to act as filling tissues in various spaces, e.g. the retromeniscal pad of tissues of the temporomandibular joint. According to the various constituents and their amounts present, the following types of connective tissues are found in the body: areolar, adipose, fibrous, elastic, reticular, and the calcified tissues cartilage and bone (see below). Neuroglia is a special connective tissue of the central nervous system with which we are not concerned in this study.

The pattern of the cells varies from type to type in the connective tissues examined, but may be listed as follows: fibroblasts, fibrocytes, macrophages, plasma cells, mast cells, and fat cells. Fibroblasts have an irregular cellular outline and cytoplasmic branches, and are closely

associated with collagen fibres (p.8.4 - 8.6); they are recognised by their pale oval nuclei with prominent nucleoli. The older fibroblasts (fibrocytes) have darker staining nuclei (some even pyknotic) which are more flattened. Macrophages are recognised by their denser, rounder nuclei and a cytoplasm which contains coarse granules and vacuoles. They may be variable in shape because of amoeboid movements. Plasma cells are either round or oval in shape; their cytoplasm is clear, and their nuclei are round and usually eccentrically placed, having chromatin arranged in a "cartwheel" appearance. Mast cells have characteristic cytoplasmic granules which stain metachromatically with toluidine blue, and their nuclei are round. Fat cells are easily recognised by their peripheral cytoplasm which is displaced by the large globule of fat it contains; their cell shape is usually polygonal due to mutual compression, and the nuclei are at the periphery of the cell, embedded in the rim of cytoplasm surrounding the fat droplet.

Intercellular matrix consists of (a) fibres, and (b) ground substance; the former appear as strands under the microscope, but the latter appears amorphous. Fibres are collagen, elastic, reticular, and oxytalan, each type having its individual characteristics. Collagen fibres (Chapter 8) are particularly relevant to the present study. They are the most widely distributed type of connective tissue fibres in the body, and are responsible for the strength and toughness of connective tissue. Elastic fibres are much thinner than collagen fibres and are not usually demonstrable by ordinary stains although orcein or resorcin fuchsin stain them well. They are easily stretched, and provide elasticity for some connective tissues, e.g.

posterior to the upper limb of the Posterior Bilaminar Zone of the human temporomandibular meniscus, where they act as active retractors of the meniscus. Reticulin fibres are closely associated with collagen fibres and exhibit a similar periodicity of 640A under the electron microscope. To demonstrate them, auric chloride is used, which stains them black. Oxytalan fibres are sometimes present in connective tissues, and are demonstrated by orcein stain after oxidation or by using aldehyde fuchsin (also requiring oxidation beforehand). Their function is unknown, although in the last decade they have been the subject of much research (Fullmer 1960, Soule 1967, Carmichael 1968, Duthy 1972).

The ground substance of connective tissue will be described in Chapter 8 (p.8.11).

All types of connective tissue contain approximately the same components but in different proportions, sometimes entirely at the expense of one or more of the other components in order to achieve specialisation. Thus in tendons and aponeuroses where great strength is required, most of the cells are fibrocytes, and most of the fibres are collagen arranged in a very dense and regular manner. Where only a passive filling function is required, there is a preponderance of ground substance (in gel or sol form), a sparse distribution of cells, and a thin and irregular arrangement of fibres.

## CARTILAGE AND BONE

Cartilage and Bone may be regarded as specialised connective tissue whose ground substance is rigid. They

give rigidity and strength, as required by the adoption of posture and movement in limbs, and the protection of certain organs such as the brain.

Both cartilage and bone have (a) cells, and (b) intercellular matrix which consists of fibres and ground substance. Essentially, therefore, they are similar to the other connective tissues in their constituents. For specific demonstration of cartilage in this study, the aldehyde fuchsin staining technique has been used, the rationale being that the intercellular matrix is rich in mucopolysaccharides (p.1.14; Appendix IX p.A.22).

CARTILAGE: The cells of cartilage are chondrocytes. Their earlier form, the chondroblasts, are associated with the production of cartilage, and are very similar in appearance to fibroblasts. As they mature, their nuclei become smaller and the nucleoli become more prominent. Their cytoplasm is pale, having either a clear appearance or showing fine granules. Chondrocytes are often found in groups of two or more, but each lies within its individual clear space (lacuna) surrounded by the gritty, stiffened intercellular matrix. Their cytoplasm often sends processes into this matrix.

The fibres in the matrix of cartilage vary according to the type of cartilage examined. In white fibrous cartilage (e.g. intervertebral disc) collagen fibres predominate; in elastic cartilage (e.g. auricle) elastic fibres are predominant, while in hyaline cartilage (e.g. tracheal rings) fibres are very limited indeed, and the extracellular matrix is almost entirely made of amorphous substance.

The ground substance of cartilage is gritty and is harder than the gel of other connective tissues because of the presence of large amounts of chondroitin sulphates and other carbohydrate-protein substances. While giving cartilage its strength, this gritty extracellular substance provides a certain degree of resiliency and elasticity.

Compared to many other tissues, cartilage is quite avascular. However, it is normally covered by a very vascular fibrous structure called the perichondrium. From this, small arterial capillaries enter the bulk of the cartilage via small canals and supply it with nutriments. Where cartilage is thin capillaries are not necessary, nutriments being able to diffuse into the interior (e.g. from the synovial cavity of a joint into the articular cartilage).

Articular cartilage on joint surfaces is important in the present study. This cartilage forms a layer on many joint surfaces (particularly in synovial joints), and functions as a shock-absorber by virtue of its slight resiliency, and at the same time provides the joint surface of the bone, which it caps, with a smooth covering for joint movements. Most articular cartilages are of the hyaline variety, but unlike many other hyaline cartilages they show no signs of calcification even late in life.

Articular cartilages have no perichondrium, but usually have a lining of synovial membrane. In this connection it may be mentioned that the fibrous covering on the condyle of the mandible does not have the characteristics

of a perichondrium, and is therefore not regarded as such; rather, it is part of the mechanism of appositional growth of the condyle, where chondrocytes are derived from the metamorphosis of the fibroblasts.

The cellular arrangement of articular cartilages is unique. In the superficial region there are flattened chondrocytes parallel to the surface, which in the deeper regions become oval or rounded, and are arranged in vertical rows perpendicular to the articular surface. In the deepest region chondrocytes are broken down and hyaline ground substance disintegrates. Here osteoblasts appear, and the hyaline ground substance is replaced by calcified cancellous bone.

BONE: Bone is a hardened connective tissue by virtue of the inorganic salts in the intercellular matrix. Its rigidity gives the body support, provides limbs with rigidity for active movements, and offers certain internal organs protection. While it is regarded normally as a hard and rigid structure, it nevertheless has a degree of resiliency which is particularly marked in young children. With age, bones become more "sclerotic" and therefore more rigid and less resilient.

Microscopically, bone is covered by a thin layer of fibrous tissue, the periosteum, which is attached to the bone surface by extensions of collagen fibres into the hard tissue, the perforating fibres of Sharpey. The outermost region of a bone is made up of dense compact bone ---

the cortical plate. This is solid, containing only very small spaces. It consists of many osteocytes (see below) and intercellular matrix, which is arranged in the form of lamellae around central Haversian canals which carry blood vessels supplying nutriments to the cells.

Interior to the compact cortical plate is cancellous bone which consists of strands of hard tissues with wide spaces for blood and nutriments. It only differs from the cortical plate by the arrangement of the cells and matrix. The direction of cancellous bony spicules are such that they provide strength and support for the bone. Thus they usually run along the lines of stress of the bone like the architectural beams of a building (e.g. in the femur). In the case of the mandibular condyle, the cancellous spicules are arranged perpendicular to the surface, giving the condyle adequate strength in all movements of the mandible like the spokes of a wheel.

The core of a bone is usually fatty tissue (fatty or yellow marrow). In some bones the marrow is made up of haemopoietic tissue --- the red marrow (e.g. the interior of the iliac crest).

The cells of bone are the osteocytes. Like cartilage cells, their earlier forms resemble fibroblasts and are called osteoblasts. Osteoblasts are associated with the formation of bone. The mature osteocyte is flattened, ovoid or almond-shaped with darkly stained nuclei. There is little cytoplasm, but it may send thin processes into the intercellular matrix. The osteocytes may be regarded as

formative cells (osteoblasts) which have been trapped in their individual lacunae as a result of the intercellular formation of matrix and deposition of inorganic salts.

The intercellular matrix of bone consists of collagen fibres and ground substance as in cartilage, but unlike cartilage there is deposition of inorganic salts thereby making bone rigid. These salts are formed from calcium, magnesium, phosphate, carbonate, chloride, fluoride, and citrate, and are mainly in the nature of calcium hydroxy-apatite --- $3(Ca_3(PO_4)_2).Ca(OH)_2$.

We shall now consider the Histology of the structures of the temporomandibular joint of the Rat.

TEMPORAL ARTICULAR SITE

Sagittally, the temporal site is a gently convex surface, and is the inferior surface of the flattened but curved temporal flange at the root of the zygoma (Fig. 1.11).

Coronally, it is in the form of an arch housing the condyle from above; its lateral wall does not extend quite as far inferiorly as the medial wall (Fig. 1.12).

From superficial to deep, the histology of the temporal articular site is divided into a COVERING and the underlying BONE.

Covering the bone is a layer of avascular fibrous tissue which is thin anteriorly but gradually thickens posteriorly to be twice as thick as that anteriorly. This fibrous

Fig. 1.11 Temporomandibular Joint, Sagittal section
H & E x 25

T = temporal site C = Condyle
AM = Anterior end (meniscus) S = Synovium

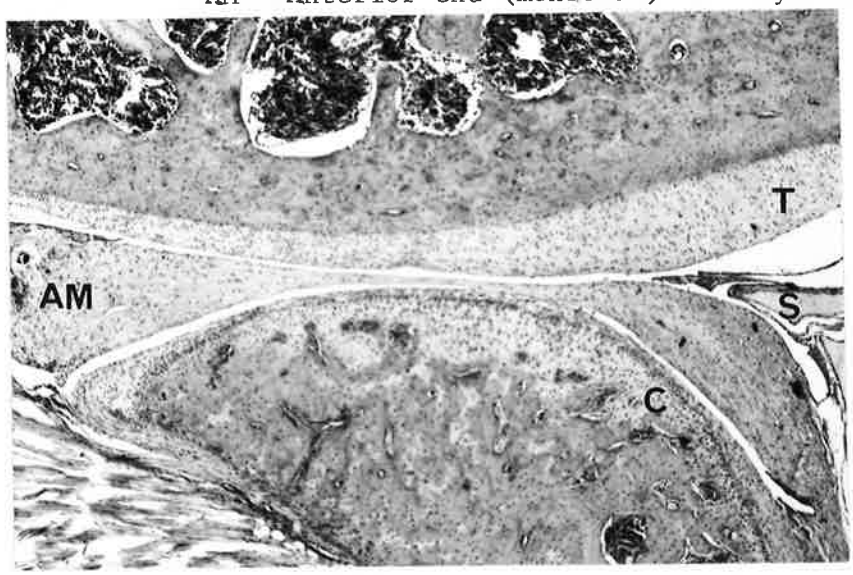
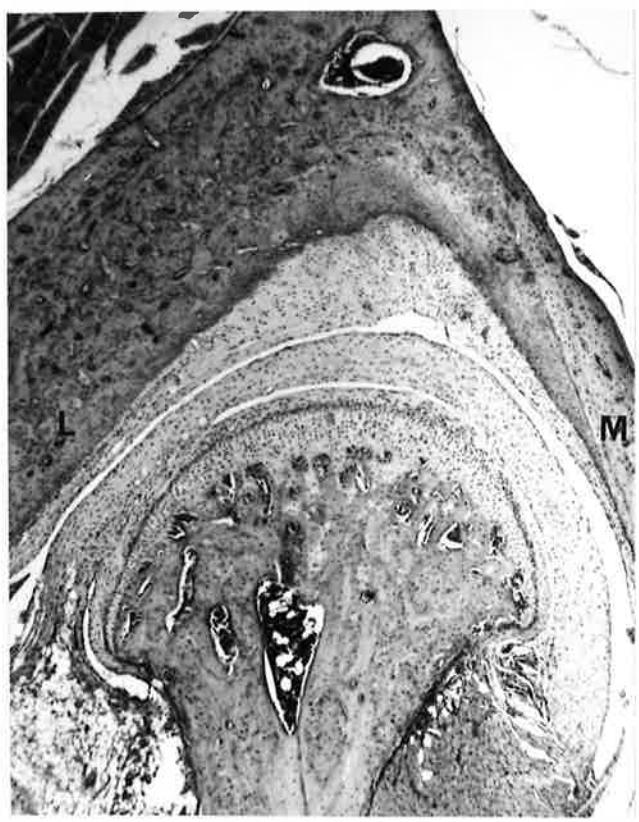



Fig. 1.12 Temporomandibular Joint, Coronal section
H & E x 25

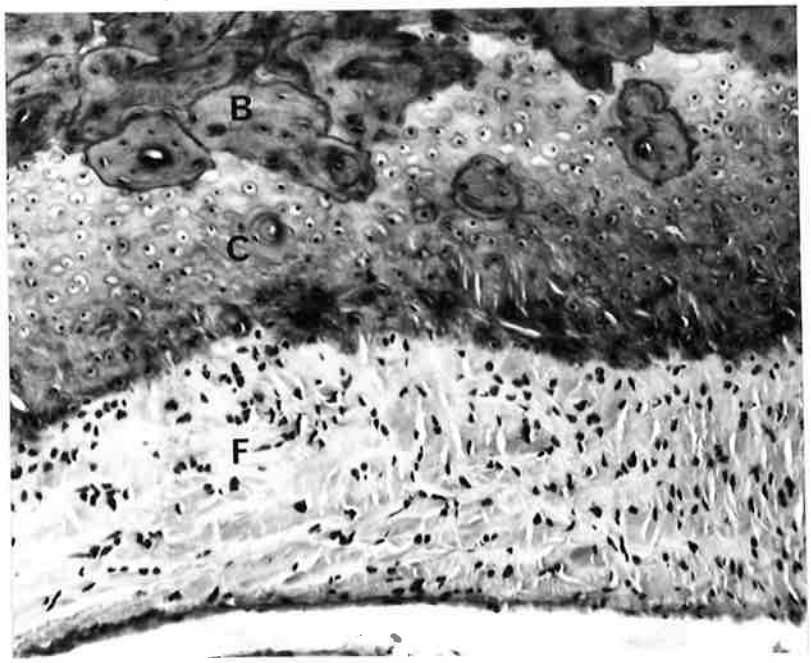
M = Medial end L = Lateral end



covering is divided into a superficial and a deep layer. The superficial layer is very thin and consists of collagen fibres running parallel to the surface with an occasional fibrocyte which is also orientated parallel to the surface. This thin layer occupies about one-tenth of the total thickness of the covering at the anterior and lateral parts of the joint, and one-twentieth at the posterior and medial part of the joint.

The deep portion of the covering consists of collagen fibres in general perpendicular to the surface, although in the posterior region the fibres vary in direction. Among the fibres are fibrocytes, fibroblasts (less mature fibrocytes), and chondrocytes adjacent to bone. The fibrocytes are distributed in columns which are perpendicular to the surface (like collagen fibres). In the thinner anterior regions of the covering the cellular distribution is denser.

Fig. 1.13 TEMPORAL SITE, Coronal section (top of temporal arch). H & E x 100




F = Fibrous covering
C = Cartilage
B = Bone

The junction of the fibrous covering and the cartilage layer is well defined in Haematoxylin and Eosin stained sections by an abrupt change in colour from pink to purple.

The covering is continuous anteriorly beyond the joint surface as a fibrocartilagenous extension of the temporal flange just posterior to the temporalis, acting as a pulley facilitating the play of this muscle over bone.

Posteriorly the covering extends upwards to leave the joint, and tapers downwards to disappear into the retromeniscal pad of loose tissues.

The temporal bony surface consists almost entirely of compact bone with osteocytes quite evenly spaced and individual cells occupying their own lacuna. Occasional marrow cavities are seen, and these are of the red haemopoietic type, and are some distance from the joint surface.

MANDIBULAR CONDYLE

Sagittally, the condyle is a semicircular structure convex upwards opposite the gentle convexity of the temporal bone (Fig. 1.11 p.1.18).

Coronally, it is a club-shaped structure placed within the arch of the temporal site but capped by the articular meniscus (Fig. 1.12 p.1.18).

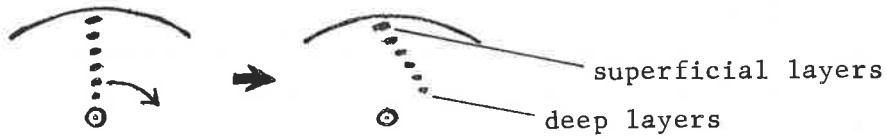
The histology of the condyle consists of a COVERING which, in its depth, is transformed into BONE.

Covering the bone superficially is first a layer of avascular fibrous tissue, which gradually is transformed into cartilage. The cartilage then merges into bone.

The fibrous layer is divided into superficial and deep parts. The superficial layer is thin, and consists of collagen fibres orientated parallel to the surface, with flattened fibrocytes orientated in the same direction.

The deep layer of the fibrous covering consists of collagen fibres which are oblique antero-posteriorly, with fibrocytes orientated in the same direction. This layer is thicker than the superficial layer. In the lateral part of the condyle the fibrous covering is thick, and is seen to wrap around the condyle until just inferior to the lateral lip of bone (Fig. 1.12 p.1.18). There it splits into an outer and an inner layer. The outer layer is loosely continuous with the articular meniscus, while the inner layer becomes the periosteum at the junction between the condyle and the ramus. Medially the fibrous covering extends beyond the medial lip downwards to be continuous with the periosteum of the condyle.

Deep to the fibrous covering is a band of fibroblasts undergoing transition into chondrocytes. This layer has about the same thickness as the deep fibrous layer just superficial to it, but the cells are all flattened and densely placed. As seen proceeding from superficial to deep, the columns of cells are oblique and give the appearance of a shearing effect such as would be produced if the most superficial layer could be kept stationary, the condyle "drags" the deeper cells when rotated forwards, creating oblique columns of transitional cells, as shown in the figure below:




The transitional zone merges into a zone of hyaline cartilage with the chondrocytes lying within the lacunae of ground substance. This layer stains positively (purple) with aldehyde fuchsin (Fig. 1.14 on p.1.23). While appositional cartilagenous growth of the condyle is seen throughout the whole of its mediolateral width, it is most active medially and only minimal laterally. At the medial pole it wraps around the bony medial lip and protrudes as a cartilagenous tip. The condyle therefore lengthens not only vertically but also medially. Because of the less active growth at the lateral region of the condyle, its general direction of lengthening is upwards and medially (Fig. 1.12).

The deepest part of the cartilagenous zone runs into areas where chondrocytes disintegrate and cartilage is being transformed. Bone is being formed here, replacing the disintegrating cartilage. The deeper areas of cartilage transformation appear to be continuous with various parts of the marrow spaces.

The bone of the mandibular condyle consists of cancellous bone with trabeculae generally perpendicular to the surface thus giving strength to the condyle. Marrow spaces are present and are of the red haemopoietic type. Anteriorly, direct insertion of the lateral pterygoid muscle into bone is seen (Fig. 1.15).

The deepest part of the condylar process is continuous with the compact bone which forms the upper part of the ascending ramus of the mandible.

Fig. 1.14 Mandibular condyle, Sagittal section.
Aldehyde fuchsin x 100

C = Cartilage layer

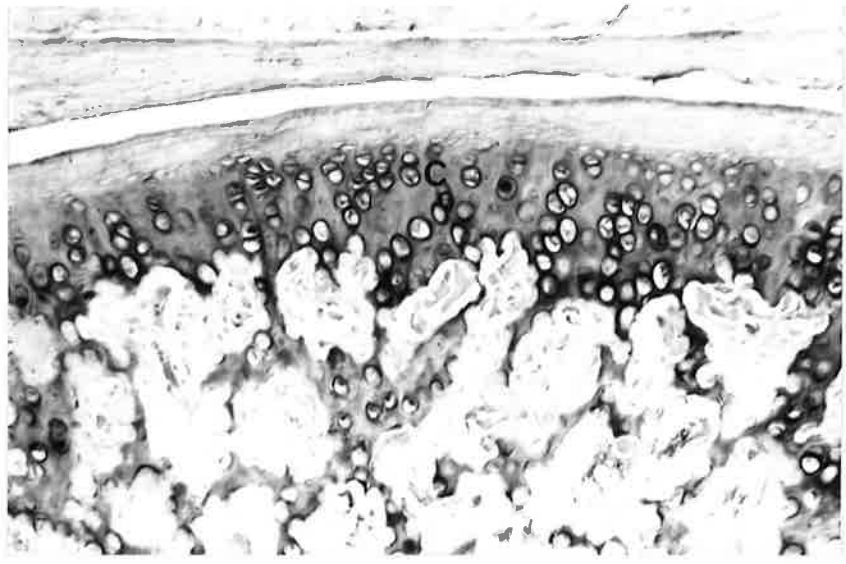



Fig. 1.15 Insertion of Lateral pterygoid muscle.
H & E x 40

L = Lateral pterygoid
C = Condyle
M = Articular Meniscus

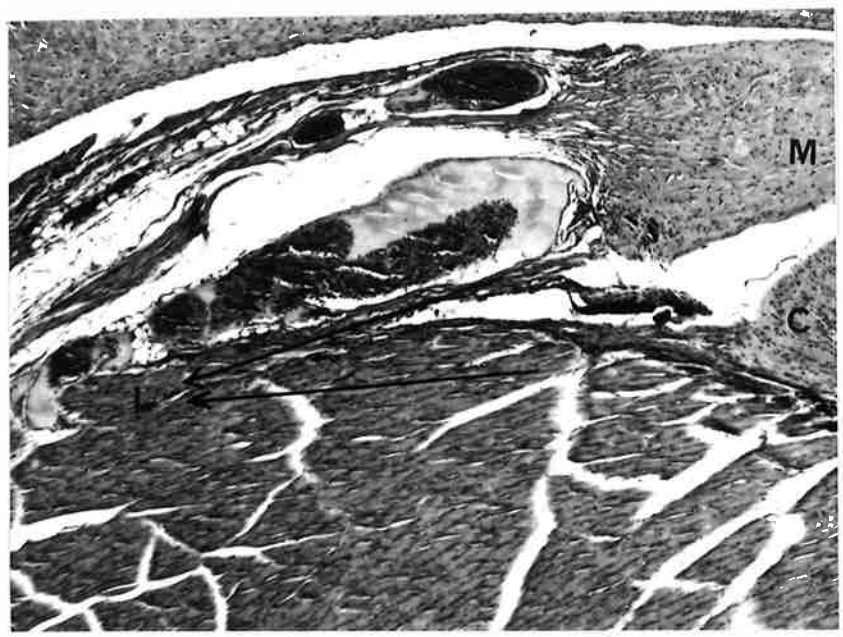
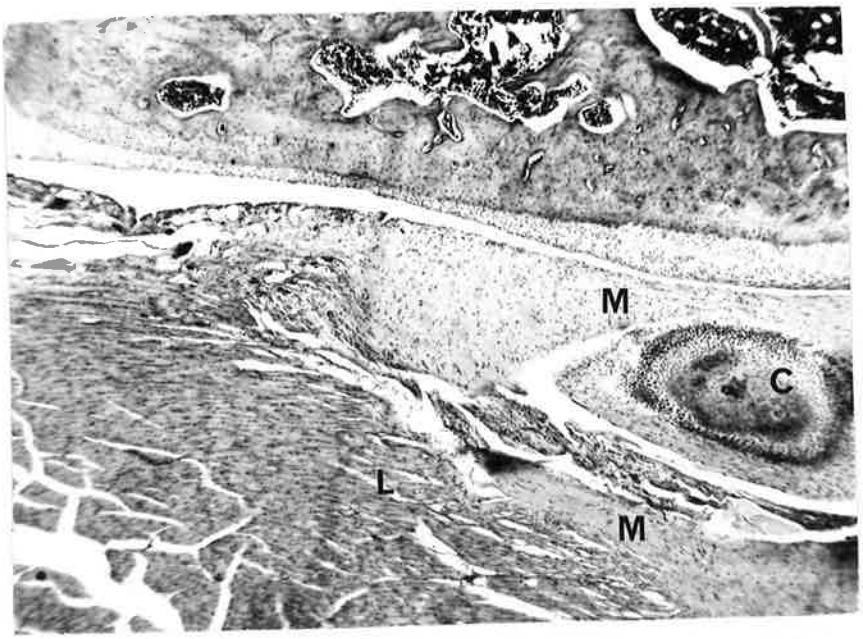


Fig. 1.16 MEDIAL REGION OF TEMPORO-MANDIBULAR JOINT, showing conclusively the attachment of lateral pterygoid muscle to the meniscus at this region.

H & E x 25

M = Meniscus
C = Condyle
L = Lateral pterygoid



## ARTICULAR MENISCUS

The articular meniscus in the sagittal plane is a biconcave structure, interposed between the temporal articular site and the condyle. Coronally it is in the form of an arc which caps the condyle (Fig. 1.11 & 1.12). In both planes its respective surfaces parallel that of the temporal and condylar surfaces. Thus where the fibrous covering of the temporal site is thick and where the fibrocartilage of the condyle is thick, the meniscus is thin, and vice versa. The meniscus is avascular, and consists of collagen bundles with many fibrocytes and fibroblasts (younger fibrocytes with larger and paler-staining nuclei). There are no chondrocytes or evidence of cartilage (as shown by the absence of mucopolysaccharides, Fig. 1.14 on p.1.23), but some less mature fibroblasts are rounded and resemble chondrocytes in shape. The meniscus has four zones, and the distribution of cells varies from zone to zone:

(1) Anterior Thick Zone (Fig. 1.11 p.1.18): On both surfaces opposite the temporal site and the mandibular condyle respectively there is a very thin layer of collagen fibres orientated parallel to the surface. Within the layer are found some flattened fibrocytes parallel to the surface also. Both of these two superficial layers are about one or two fibrocytes thick, and are very thin relative to the thickness of the zone. The remainder of the thickness of the zone, which is its main substance, consists of collagen bundles running in different directions. Scattered among this three dimensional structure are fibroblasts and fibrocytes which are distributed at even distances from one another, there being a tendency for more fibroblasts at the posterior

region and more fibrocytes anteriorly.

Anteriorly, this thick zone is continuous with a loose vascular body made up of small blood vessels, a lymph vessel, and adipose tissue. Through this body the meniscus is continuous with the attachment of some fibres of the lateral pterygoid muscle (Fig. 1.15). This attachment is only seen on the medial part of the meniscus; laterally the lateral pterygoid is attached entirely to the condylar head and neck (Fig. 1.11).

The superior surface of the Anterior thick zone extends a long way before becoming recurrent, attaching itself loosely to the pre-temporal cartilagenous "pulley" for the temporalis muscle, while the inferior surface extends for a shorter distance forwards before recurring to be continuous with the superficial layer of the fibrous covering of the condyle.

Posteriorly, this thick zone becomes thinner gradually, tapering to be continuous with the Intermediate thin zone of the meniscus.

(2) Intermediate Thin Zone: This is the zone created by the tapering of the Anterior and Posterior thick zones where the main substance of the multi-directional collagen bundles is lost. It consists only, therefore, of collagen fibres running parallel to the surfaces, with flattened fibrocytes and fibroblasts also orientated parallel to the surfaces. The thickness of this zone is only one-sixth of the maximum thickness of the whole meniscus.

(3) Posterior Thick Zone: In shape and structure the Posterior thick zone is a mirror image of the Anterior thick zone. The superficial thin fibrous layers and the thick multi-directional collagen comprising the main substance have the same arrangement as the Anterior thick zone. More fibroblasts are found near the Intermediate thin zone, while more fibrocytes are found near the thick end. Hence, there are more rounded cells near the thin Intermediate zone anteriorly and posteriorly, and more mature flattened cells near the anterior and posterior extremities of the meniscus.

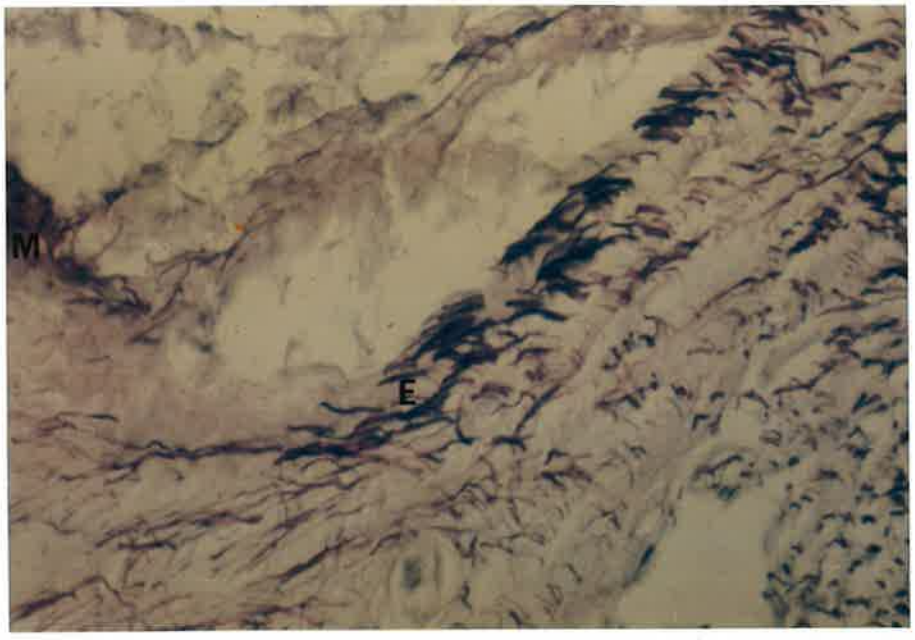
(4) Posterior Bilaminar Zone: The posterior end of the Posterior thick zone is continuous with this zone which may be divided into an ascending portion and a descending portion. The ascending portion is continuous with the loose tissues and the synovial membrane of the Joint. The synovial membrane folds upon itself several times before attaching loosely to the superficial covering of the temporal bone. The descending portion of the Bilaminar Zone consists of slightly denser interlacing collagen fibres and runs a straighter course, being parallel to and curving with the posterior slope of the condyle. The attachment to the posterior aspect of the condyle is broad but loose and indefinite. Both laminae are provided with many elastic fibres at their posterior extremities --- these fibres probably act as active meniscal retractors when the lateral pterygoid muscle relaxes (Fig. 1.17 on p.1.28) (Christensen 1969).

The Posterior Bilaminar Zone merges into the retromeniscal pad of loose areolar tissue which is rich in blood vessels.

Fig. 1.17 Elastic fibres at upper (ascending) limb of Posterior Bilaminar Zone.

Aldehyde fuchsin x 250

M = Meniscus
E = Elastic fibres (darkly stained)




Medially, the meniscus extends just inferior to the medial lip of the condyle and is seen to be attached to the medial part of the lateral pterygoid muscle. There is no fibrous capsule of the joint.

Laterally, the meniscus extends below the lateral lip of the condyle, and terminates rather abruptly by splitting into an outer and an inner part. The outer part is continuous with the very thin deep fascia of the masseter, while the inner part becomes loose, and is continuous with some areolar tissue before ending in the periosteum of the ramus. Again, there is no anatomically distinguishable fibrous capsule of the joint (Fig. 1.12 on p.1.18).

## FUNCTIONAL ANATOMY AND HISTOPHYSIOLOGY OF THE RAT TEMPOROMANDIBULAR JOINT STRUCTURES

The masticatory pattern of the Rat consists of (1) opening and closing of the mandible for incision, and (2) antero-posterior excursions of the mandible for grinding of food. For incision, the flattened convexity of the temporal bone allows the convex menisco-condylar complex to rotate on it with ease; for antero-posterior grinding, it can be seen that the convex condyle can move without impediment on the broad convex temporal surface.

The temporal gutter is no impediment to the condylar movements required in the masticatory pattern of the rat. What the gutter prevents is lateral condylar rotation such as that which occurs in the sheep (Lam 1971). The effect of the gutter is to promote functional efficiency during anteroposterior excursions by limiting lateral play of the condyle within it.

The articular meniscus compensates for discrepancies in the curvature of the temporal site and the condyle during all phases of mandibular movements, as is evident from its "parallel" outlines which follow those of the articular surfaces. The mode of its attachment to the temporal site and the condyle allows it to travel anteriorly and posteriorly with the condyle while at the same time allowing the latter to rotate on it in the sagittal plane. It also allows the whole menisco-condylar complex to slide on the temporal surface. As the whole complex slides anteriorly, the folded synovium and the loose

retromeniscal tissues allow plenty of yield, with the loose attachment of the meniscus with the temporal bone having the same effect anteriorly.

To prevent the condyle from dragging the meniscus against the temporal site during its excursions, the meniscus is provided with an active protractor made up of some fibres from the lateral pterygoid muscle (Fig. 2.14 on p.2.15, and Fig. 1.15 on p.1.23).

The temporal articular site and the mandibular condyle are both covered by avascular fibrocartilage as these are the areas subjected to some masticatory pressure. Immediately beyond the anterior limit of the joint (i.e. outside of pressure areas), the articular meniscus is found to be continuous with vascular tissue.

No fibrous capsule equivalent to that of the human temporomandibular joint was observed in the rat. This is no doubt due to the fact that because the temporal gutter can adequately limit the mediolateral displacements of the condyle, no further limitation by a fibrous capsule is necessary. Anteriorly, therefore, the condyle can travel a long way unrestricted, but posteriorly it is limited by the acoustic meatus beyond the retromeniscal pad of loose tissues.

# CHAPTER 2

## FUNCTIONALLY RELATED STRUCTURES OF THE TEMPOROMANDIBULAR JOINT

The functionally related structures of the temporomandibular joint of the rat are: (1) the muscles of mastication, and (2) the teeth. Consideration will be given to these structures in this discussion in so far as they are related to function of the joint.

## MUSCLES OF MASTICATION

To appreciate the functional role of the muscles of mastication, an understanding of the principles underlying muscle action is necessary.

### PRINCIPLES OF MUSCLE ACTION

The muscles of mastication belong to the class of striated muscles, which are so called because their fibres show transverse striations (the A, H, I, Z Bands) when examined under the light microscope.

MUSCLE SIZE: The size of a muscle is determined by the number of muscle fibres it contains, and by the individual size of the fibres themselves. The size is directly related to its power, a larger muscle affording more power than a smaller one. In Man, the gluteus maximus lifts the weight of the body in rising from the sitting position, and is therefore found to be much larger in size than the lateral pterygoid which acts only in a less powerful way on the mandible.

MUSCLE FORM: Broad and stout muscles are designed for power, while long and thin muscles are designed for translatory movement. In Man, the broad pectoralis major belongs to the former type, while the extensor indicis belongs to the latter.

MUSCLE ACTION: A muscle may contract isotonically or isometrically. Isotonic contraction causes shortening and therefore movement of the structures to which the muscle is attached. Usually one end of a muscle is fixed and when acting causes the other end to move; the attachment area of the fixed end is called the "origin" of the muscle, while that of the moving end is the "insertion". In isotonic contraction, the insertion of the muscle may be the site of maximum movement, such as the anterior belly of the digastric muscle, in which case maximum efficiency is afforded by the power of the muscle. On the other hand, the insertion may be distant from the site of maximum movement, such as the biceps brachii, in which case some power is sacrificed for the sake of producing more movement by leverage.

Isometric contraction increases intramuscular tension without causing any movement. In this case power is used only for steadying the actions of other muscles (such as steadying of the hyoid bone by the infrahyoid muscles for the anterior belly of the digastric), and in preventing movements such as in the maintenance of posture and in acting against resistance.

Muscles where attachments are "fixed-to-fixed" (e.g. temporalis) may carry out one or both of the above types of contraction. With muscles of the "fixed-to-loose" type (e.g. muscles of facial expression) only isotonic contraction is possible because the loose end always moves on contraction.

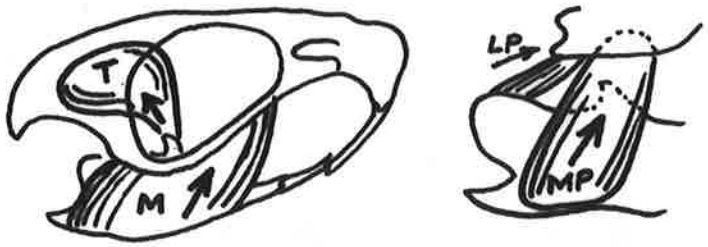
TENDONS, APONEUROSES: Tendons and aponeuroses are white fibrous structures made up of collagen fibres. They are found attached to many muscles, and in many instances provide a "tendinous" or "aponeurotic" insertion of muscles to bone. One of their functions is to concentrate the power of the whole muscle onto the point of application, whatever the shape of the muscle. The force can therefore be focused on a small area while the muscle itself can be extensive in origin and hence large enough to provide great strength, e.g. the deltoid, temporalis. A second function of tendons and aponeuroses is to act as extensions of muscle fibres into their insertion. This enables muscle fibres to be shorter, economising on energy expenditure, while giving the same range of movement because tendons and aponeuroses are not stretchable.

MUSCULAR CO-ORDINATION: The action of one muscle is always accompanied by some action of others in the area. Movement at the site of insertion often requires fixation of the site of origin. The prime mover of such a movement is steadied by a group of "antagonists". Antagonists are usually just as important in a movement as the prime movers of that movement, and are necessary for smooth and steady action. At the end of each movement, the antagonists contract more strongly than the prime movers, bringing the movement to a stop. A concept of integrated, co-ordinated muscular action is therefore important in understanding all movements of the skeleton.

This concept is applicable in the same way to a muscle with many heads which act in different directions, and in which the heads vary in size and shape. When dealing with the function of such a muscle, not only is the direction of

its various heads important, but also their size and shape, and their relationship with each other.

Fig. 2.1 THE MASTICATORY MUSCLES OF THE RAT




**M = Masseter** **MP = Med.pter.**
**T = Temporalis** **LP = Lat.pter.**

MASSETER

The masseter is massive and has four heads: Superficial, Infraorbital, Intermediate, and Deep. Its outline is a bulky triangle with apex anteriorly, extending from the zygomatic arch to the lateral aspect of the ramus of the mandible.

(I) SUPERFICIAL HEAD: This arises from the outer aspect of the most anterior part of the zygomatic arch as a flat and shiny tendon. The fibres fan out as they travel horizontally backwards with a slight downward incline. They are inserted into the lowermost portion of the inferior border of the mandibular ramus, and, sweeping around it, unite with the medial pterygoid muscle at the pterygo-masseteric sling (Fig. 2.2 on p.2.5). Separation of this head from the Intermediate head is only possible anteriorly because of the tendon; posteriorly the Superficial head merges into the Intermediate head.

Fig. 2.2 MASSETER, IN SITU (Right side)

M = Masseter T = Temporalis
E = Eye A = Auditory canal

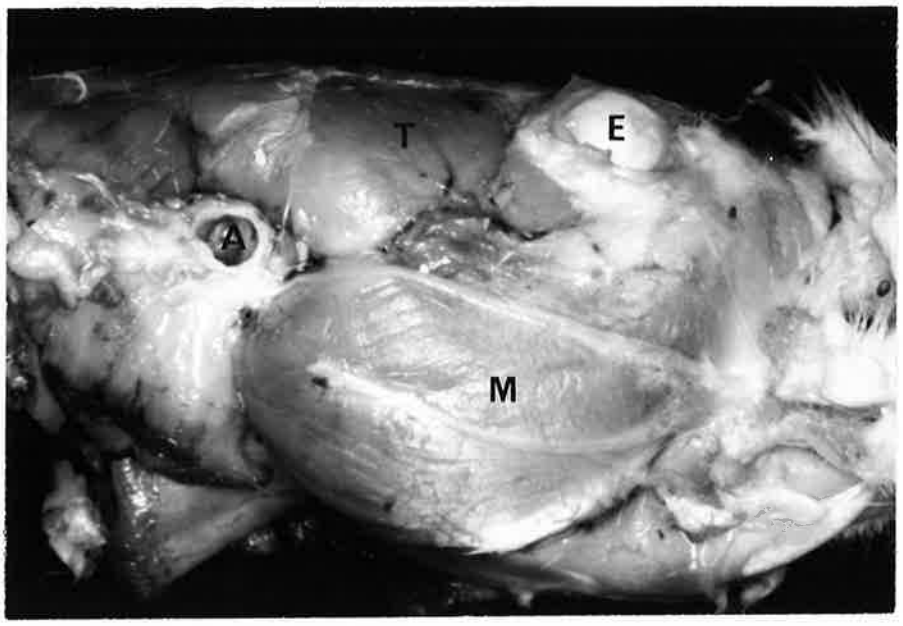
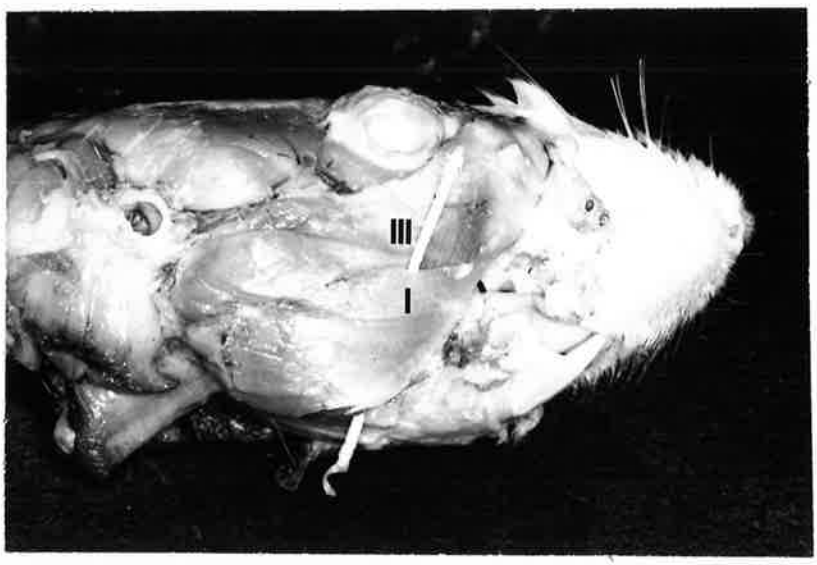



Fig. 2.3 MASSETER, Superficial and Intermediate heads separated

I = Superficial head
III = Intermediate head



(II) INFRAORBITAL HEAD: This is a small band arising from the medial wall and floor of the infraorbital foramen as fleshy fibres and travelling postero-inferiorly lateral to the mandibular ramus. It ends in an aponeurosis which is inserted into the anteroinferior area of the ramus (Fig. 2.4 on p.2.7). Its size is small compared to the Superficial head and it may be regarded as a stabilizer of the mandible.

(III) INTERMEDIATE HEAD: This extensive head is so named because of its intermediate depth between the Superficial and Deep heads. All its fibres travel parallel to one another in a postero-inferior direction. It arises from the whole of the inferior surface of the zygomatic arch with the exception of the most posterior 2-3mm, and inserts into a wide area on the lateral aspect of the ramus (Fig. 2.5 on p.2.7). The posterior part of its origin is covered laterally by an aponeurosis.

(IV) DEEP HEAD: This is a very small head originating from the most posterior 2-3mm of the inferior surface of the zygomatic arch just lateral to the temporo-mandibular joint. The fibres run antero-inferiorly and, coverging slightly, insert into a small area on the lateral aspect of the ramus just inferior to the head of the condyle (Fig. 2.6 on p.2.8).

Actions: The various heads of the masseter provide a good example of a multi-directional unit (Fig. 2.7 on p.2.8). Its physical size and direction of action reflect its functional role. The main action of the masseter is to provide a forward pull on the mandible by the Superficial

Fig. 2.4 MASSETER, Infraorbital head.

II = Infraorbital head

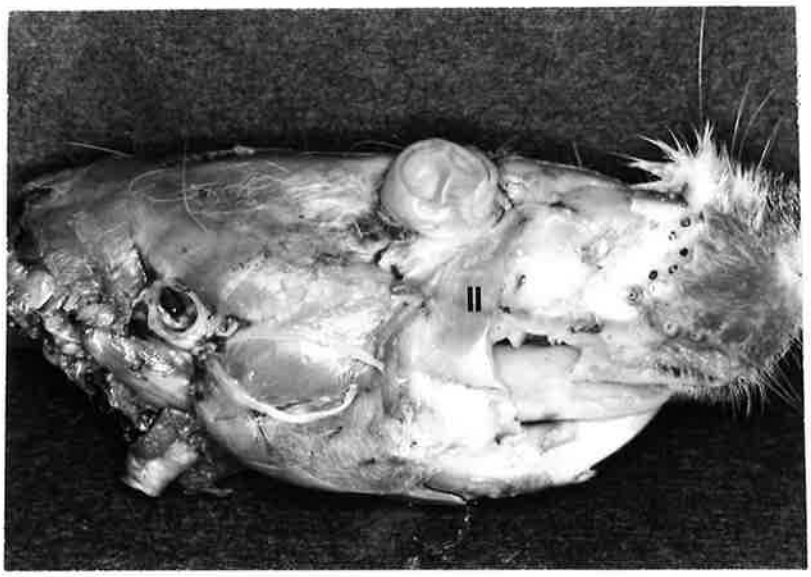



Fig. 2.5 MASSETER, Intermediate head (Superficial head reflected)

III = Intermediate head

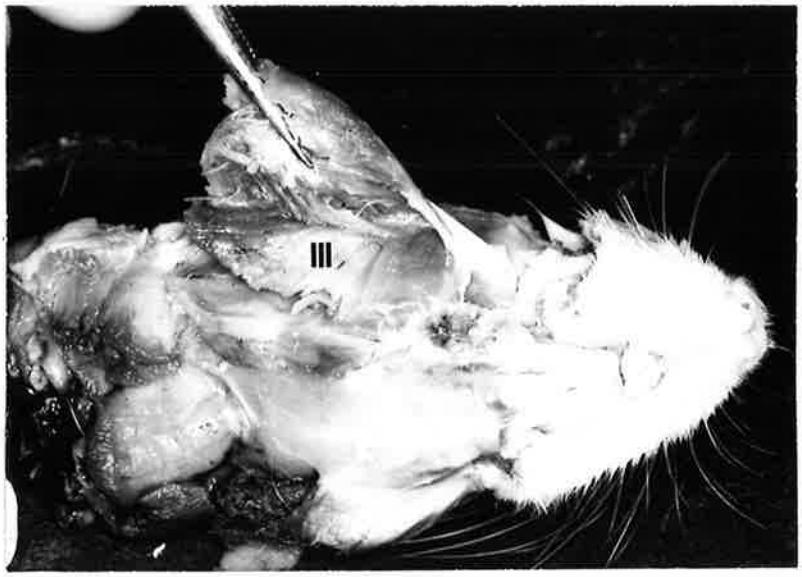


Fig. 2.6 RAT SKULL (Right side), showing attachments of heads of masseter.

I = Superficial head
II = Infraorbital head
III = Intermediate head
IV = Deep head

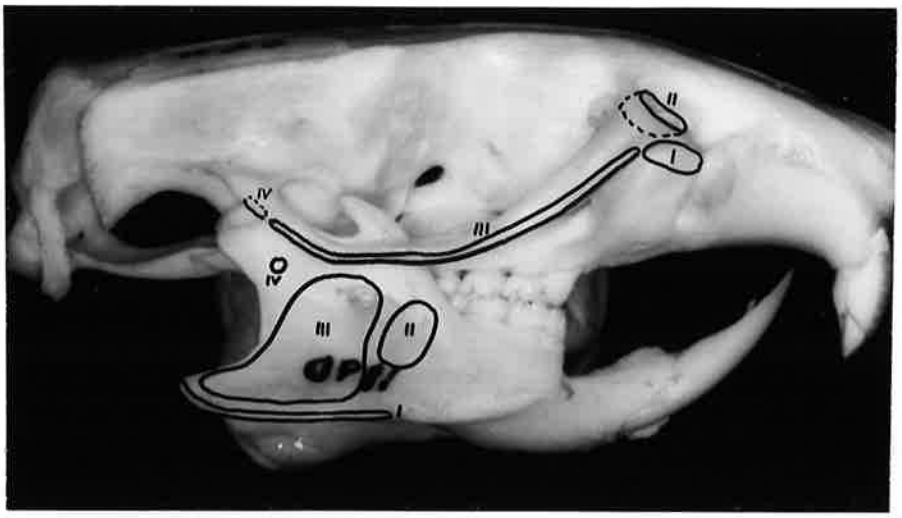
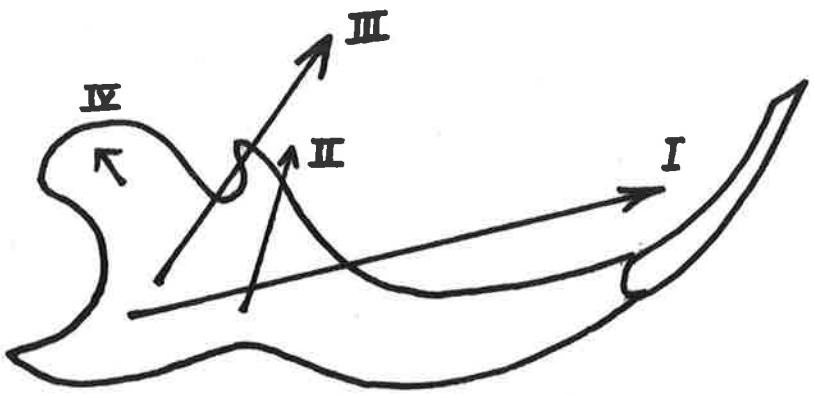



Fig. 2.7 THE COMPONENTS OF MASSETER



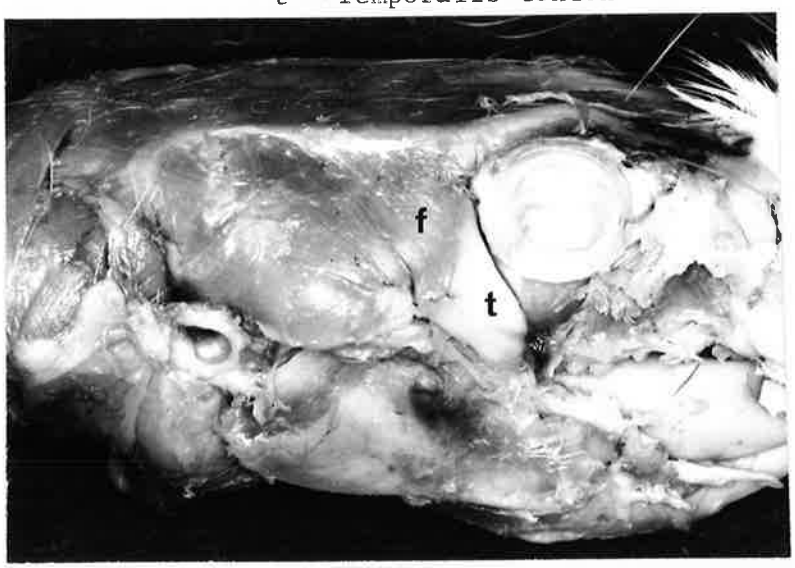
head, and an antero-superior pull by the Intermediate head. These actions are used in chewing and in promoting mandibular closure. The Infraorbital and the Deep heads are small. They assist closure of the mandible by their upward pull, and act as stabilisers during chewing.

TEMPORALIS

The temporalis is a thick muscle arising from the whole of the temporal fossa on the lateral aspect of the temporal bone. Anteriorly the origin extends downwards to just lateral to the infraorbital fissure as the Deep Part.

The fibres originating from the temporal fossa converge as they travel anteriorly to form a stout tendon which plays over a fibrocartilagenous "pulley" at the root of the zygomatic arch. The tendon inserts into the coronoid process of the mandible and downwards along the anterior border of the ramus to the level of the molar teeth. Its insertion is well demarcated from that of the masseter by a ridge (Fig. 2.8).

Fig. 2.8 TEMPORALIS (Right side)
f = Temporalis fibres
t = Temporalis tendon



The Deep Part of the temporalis originates from the infratemporal fossa lateral to the infraorbital fissure and travels parallel to the temporalis tendon as fleshy fibres throughout its course. It is inserted into the anterior part of the medial aspect of the ramus of the mandible (Fig. 2.9).

Action: The temporalis is an elevator of the mandible, and is used in incision and in maintaining the teeth in contact during the chewing of food.

Fig. 2.9 TEMPORALIS, Deep Part.

D = Deep Part
R = Mandibular ramus
T = Temporal fossa

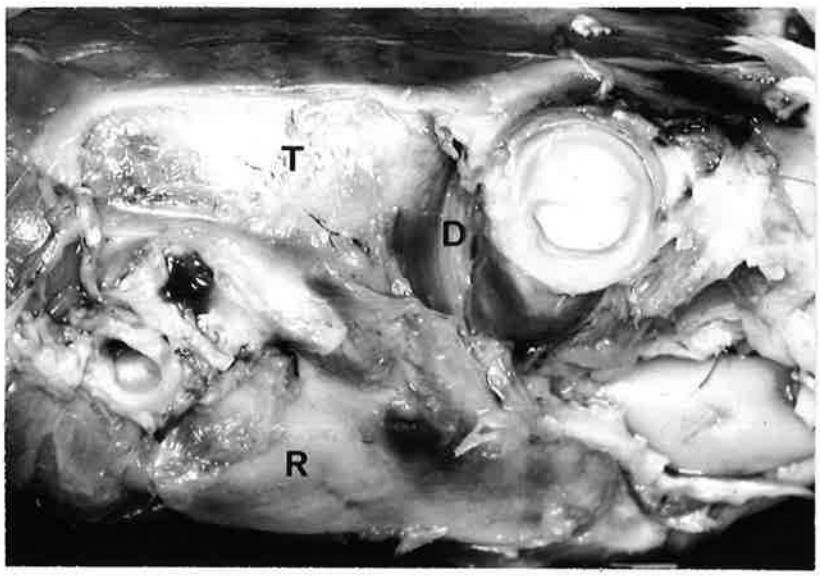
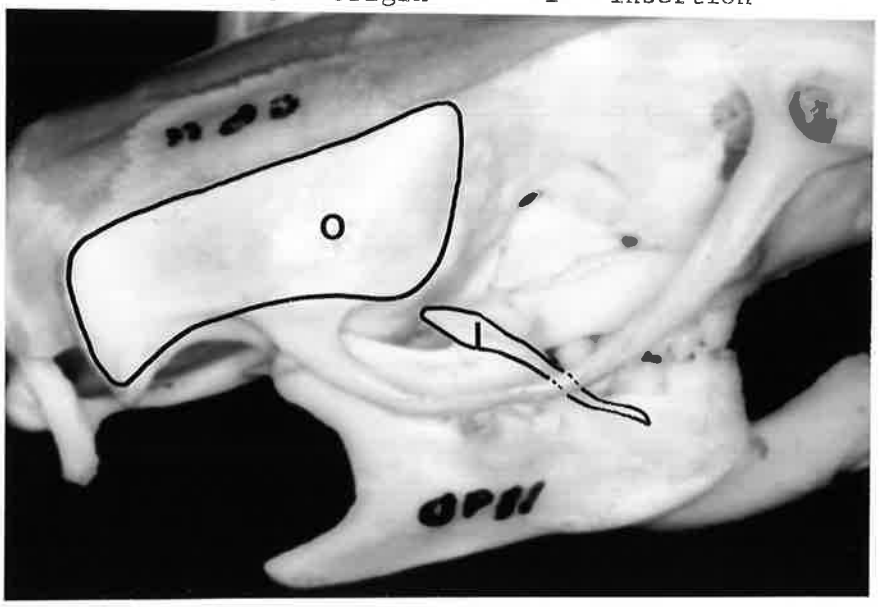


Fig. 2.10 RAT SKULL, showing attachments of temporalis (supero-posterior view of Right side)

O = Origin I = Insertion




MEDIAL PTERYGOID

The medial pterygoid is a smaller counterpart of the masseter on the medial side of the ramus of the mandible. It has a fleshy origin from the pterygoid fossa between the two pterygoid plates. It fans out postero-inferiorly to be inserted into the broad arrowhead-shaped fossa on the medial aspect of the ramus (Fig. 2.11 on p.2.12). Its insertion is below the mandibular foramen, well demarcated from that of the lateral pterygoid muscle by a ridge, and from the posterior origin of the mylohyoid muscle by the downward extension of that ridge.

Action: It is a synergist of the masseter, pulling the mandible upwards and forwards, and is used in mandibular closure and in anteroposterior excursions.

Fig. 2.11 MEDIAL PTERYGOID (viewed from below)

P = Pharynx
LB = Lower border of mandible
M = Medial pterygoid plate
MP = Medial pterygoid

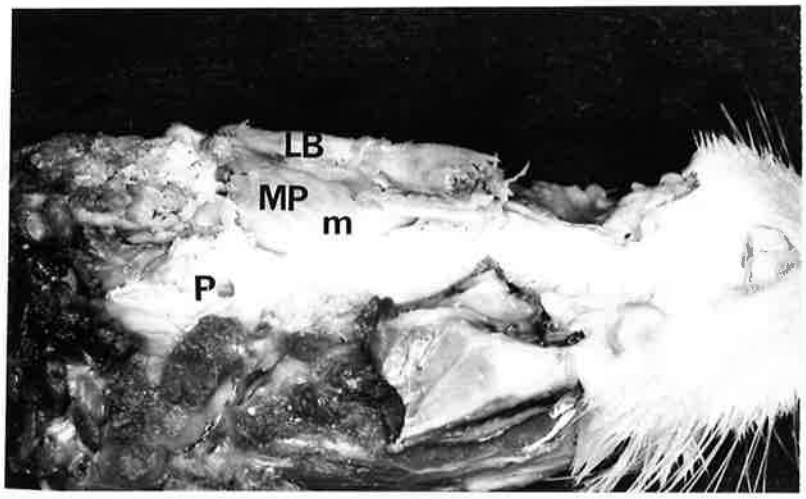
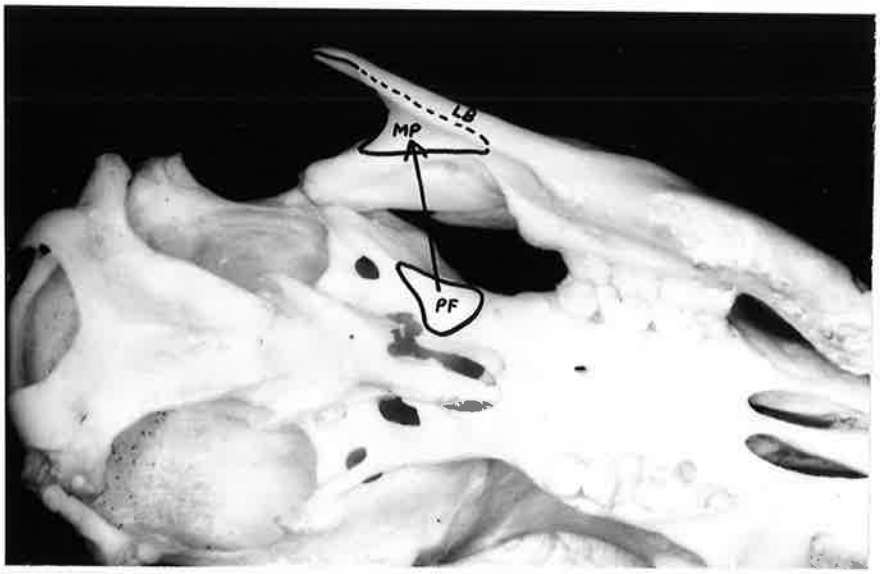



Fig. 2.12 RAT SKULL (viewed from below)

PF = Pterygoid fossa
MP = Medial pterygoid
LB = Lower border of mandible



## LATERAL PTERYGOID

The lateral pterygoid muscle is the smallest of the masticatory muscles. It has an oblong origin, with the axis antero-posteriorly, on the lateral aspect of the lateral pterygoid plate. Its fibres are fleshy throughout its course, and run parallel to one another supero-postero-medially. It is inserted into: (1) the mandibular condyle at the medial lip; (2) an area on the medial aspect of the condylar process above the mandibular foramen, and (3) the antero-medial portion of the articular meniscus of the temporomandibular joint (Figs. 2.13-2.15; Fig. 1.15 on p.1.23, and Fig. 1.16 on p.1.24).

Actions: Contraction of the lateral pterygoid produces anterior mandibular movement by its action on the condylar process, and it is therefore synergistic with the masseter in this respect. Its downward action helps the anterior belly of the digastric muscle in rotation of the mandible during opening. The part inserted into the articular meniscus of the joint moves the meniscus during mastication thereby regulating its compensating effect between the articular surfaces during such movements (see also p.4.4).

Thus, while only having one origin, the lateral pterygoid muscle is actually split into two components: one component being inserted into the mandible (condyle) and exerting a separate action from the other component which is inserted into the articular meniscus. As we shall see later, these separate actions have a functional significance (p.4.4-4.5).

Fig. 2.13 LATERAL PTERYGOID, attachment to mandibular condyle.
(Viewed from medially, anterior on observer's left)

L = Lateral pterygoid
R = Ramus of mandible

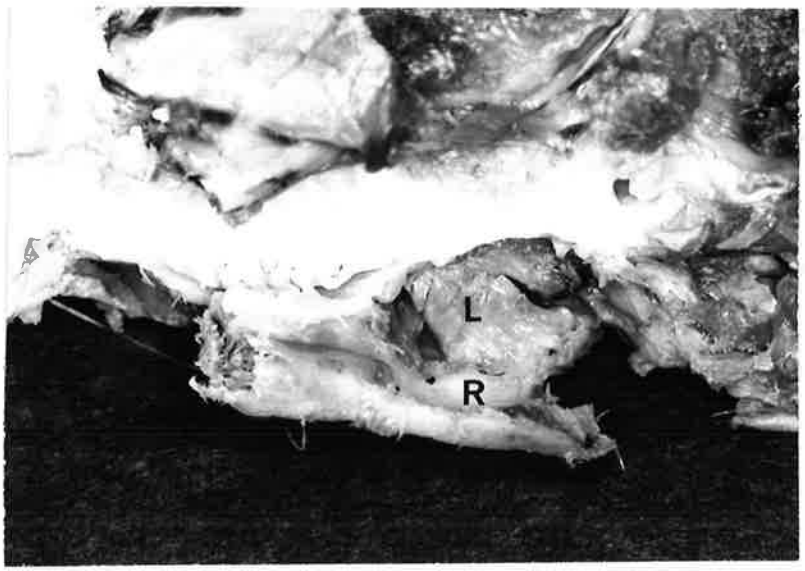


Fig. 2.14 Lateral pterygoid, attachment to articular meniscus (Right side)

L = Lateral pterygoid
C = Condylar process of mandible
M = Meniscus

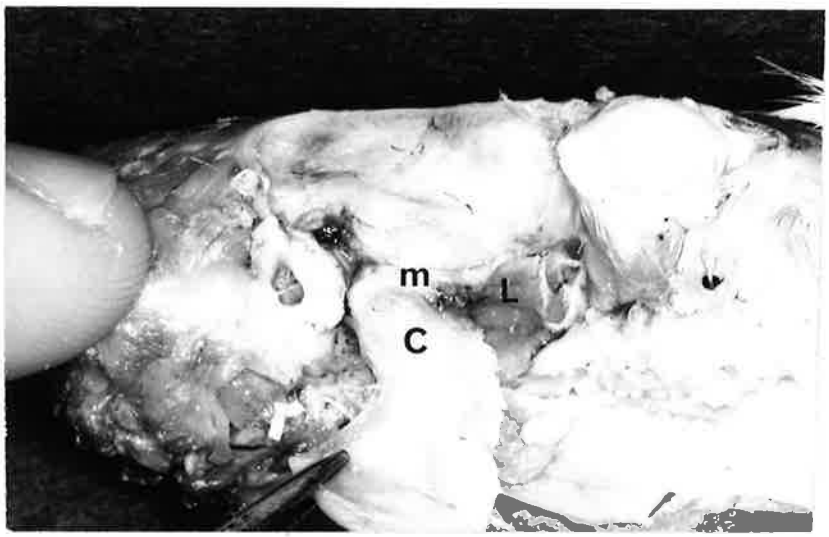
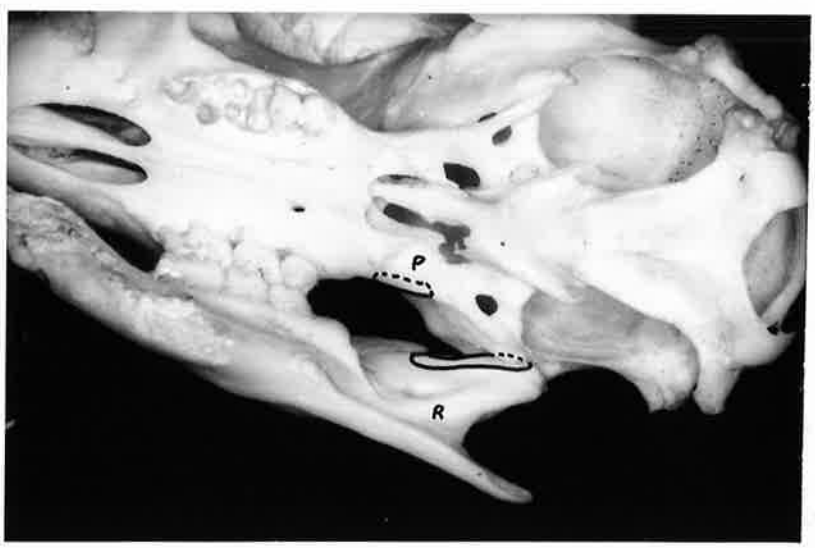



Fig. 2.15 Rat skull showing attachment of lateral pterygoid. (Viewed from below)

R = Ramus
P = Pterygoid fossa

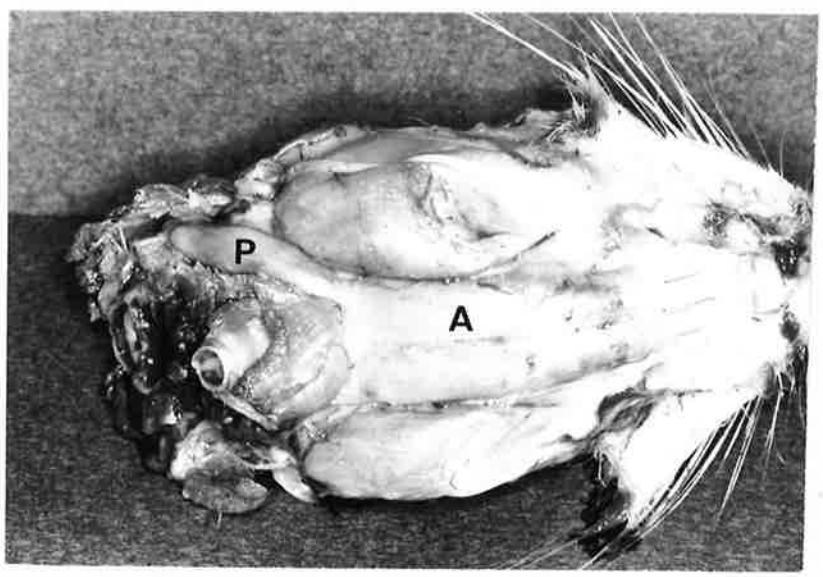


## DIGASTRIC, ANTERIOR BELLY

The anterior belly of the digastric muscle is an oblong muscle superficial inferiorly. It arises from the digastric fossa of the medial surface of the mandibular body, and runs posteriorly to be inserted into the intermediate tendon attached to the hyoid bone. It is long and fleshy, and for most of the distance is in contact with its fellow of the opposite side.

Because of the relationship of the hyoid bone to the head, the anterior belly of the digastric runs almost completely horizontally backwards, with only a slight downward incline (Fig. 2.16). This direction is different from that of the Primates where the direction is postero-inferior.

Fig. 2.16 Digastric, anterior belly and posterior bellies displayed (viewed from below).
A = Anterior belly
P = Posterior belly



Actions: (1) It retracts the mandible during anteroposterior excursions (in chewing), acting alternately and in conjunction with the anterior movers of the mandible (masseter, medial and lateral pterygoids); (2) it couples with the lateral pterygoid in mandibular opening.

## COMBINED ACTION OF THE MASTICATORY MUSCLES

The concept of a combined action of all the masticatory muscles is best illustrated by a diagram:

Fig. 2.17 COMBINED MUSCLE ACTION.


The masseter is the prime anterior mover of the mandible, the temporalis the prime elevator, and the anterior belly of the digastric the prime retractor. The lateral pterygoid and the anterior digastric produce opening.

The medial pterygoid helps the masseter both in anterior movement and in elevation, and helps the temporalis in elevation. The lateral pterygoid is synergistic with the masseter in anterior movements only.

During antero-posterior movements of the mandible in gnawing and chewing, all upward actions of the various

muscles act as vertical stabilisers, essential in producing effective horizontal movements.

## THE TEETH AND OCCLUSION

The teeth and their occlusal relationships play an extremely important role in the function of a masticatory system. In fact the role of the temporomandibular joint and the masticatory muscles is to promote the proper function of the teeth which are the preparers of food.

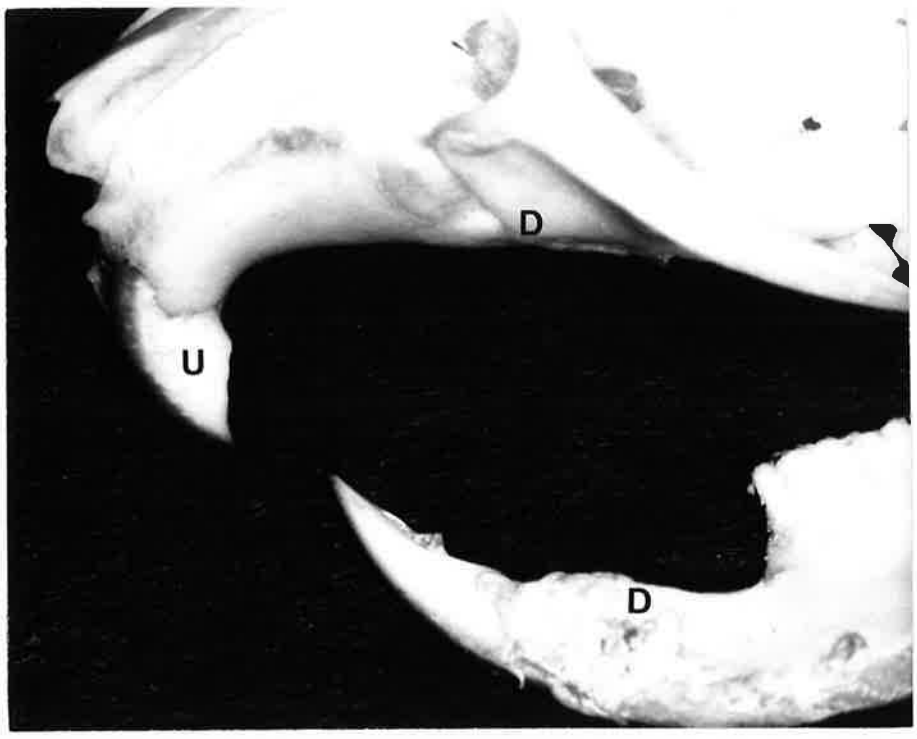
In each of the quadrants of the mouth the rat has an incisor and three molars. Its dental formula is therefore I1 M3.

There is one pair of incisors in each jaw, and hence the rat belongs to the Order Simplicidentata. The incisor has enamel only on its labial surface, which with constant wear maintains a sharp cutting edge at all times. The incisor is used not only for incising food, but also for gnawing objects, e.g. cutting through a lead pipe. The incisor wears away constantly, at a rate of up to several millimetres in a week, and this wear is continually replaced by eruption and rapid deposition of non-tubular secondary dentine in the pulp chamber (Young 1962). The incisors are large and curved, and have a steep wear facet which fits in with the closing excursions of the mandible during biting and gnawing (Fig. 2.18).

Between the incisor and the molars is a very wide diastema, into which buccal mucosa can fold, thereby

Fig. 2.18 Steep wear facets on Incisors

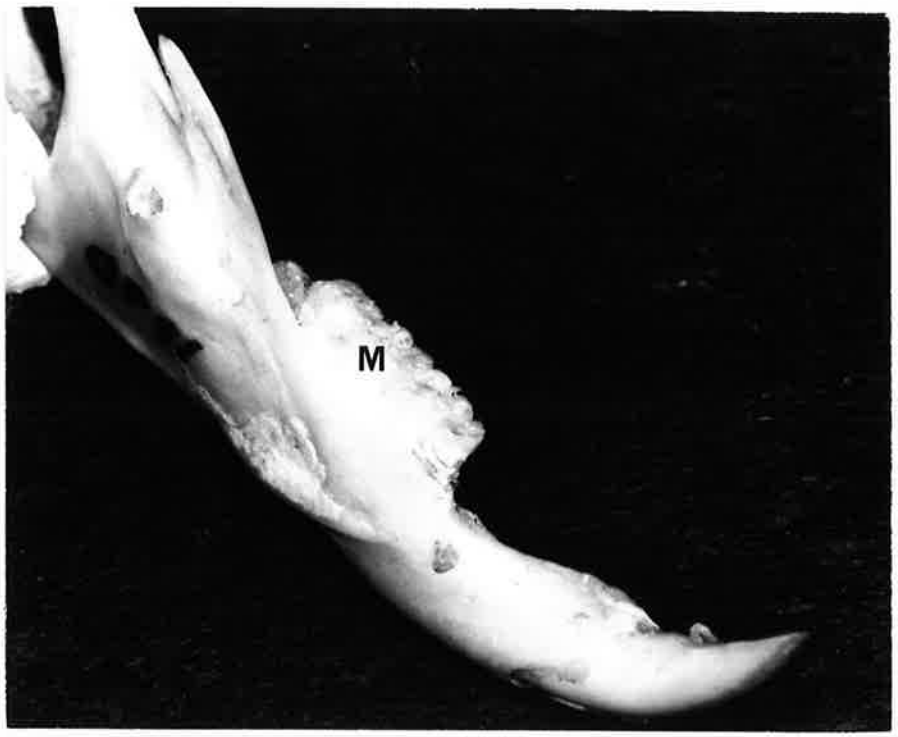
U = Upper central incisor
D = Diastema between incisor and molars




dividing the oral cavity into an anterior and a posterior chamber. Substances gnawed by the incisors therefore do not need to be swallowed.

The three molars are adjacent to one another. The occlusal morphology is made up of cross-ridges giving rise to "multi-lophodont" molars. The roots of these teeth are also wide open and erupt continually like the incisors. The occlusal surfaces show flattening due to wear, and the facets indicate grinding in the horizontal plane (Fig. 2.19 on p.2.20).

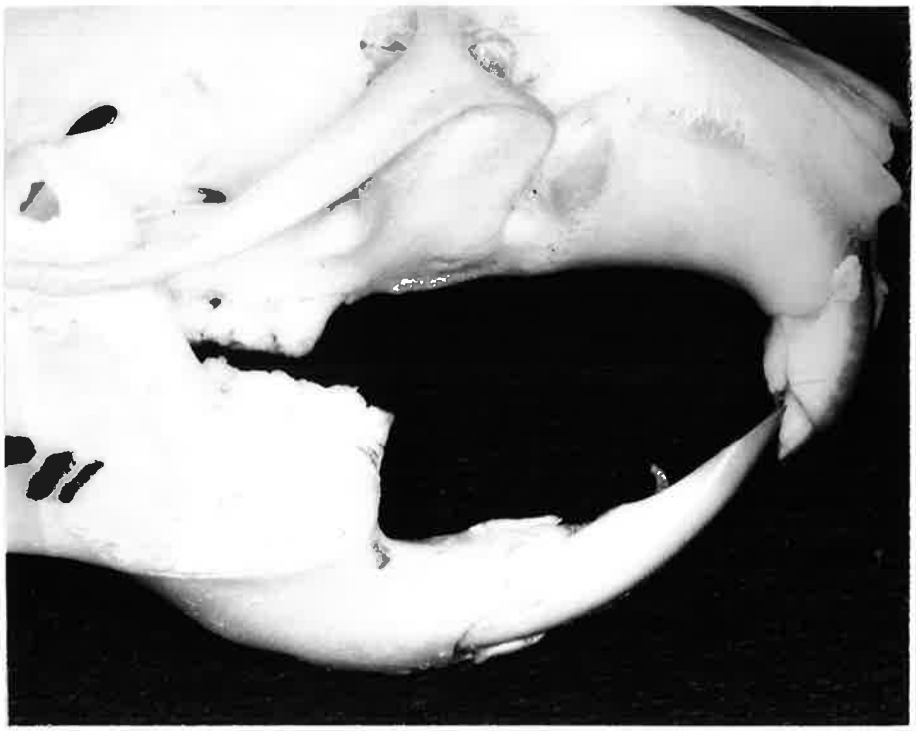
Fig. 2.19 Flat wear facets on molars
M = Mandibular second molar




The occlusion is interesting in that not all the teeth can be made to occlude at the same time. In the "intercuspal" position of the mandible, the upper and lower molars are in occlusion but the upper and lower incisors do not occlude. The molars meet in cross-bite, viz. the lower arch is broader than the upper.

When the incisors are brought together during gnawing, the mandible is moved so far anteriorly that the molars are no longer in occlusion (Fig. 2.20).

Fig. 2.20 Molars out of occlusion when the incisors are in contact.

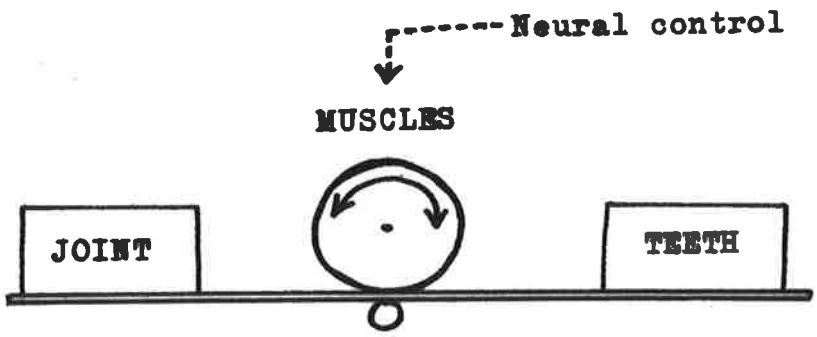


# CHAPTER 3

## THE ROLE OF THE TEMPOROMANDIBULAR JOINT : FUNCTIONAL OCCLUSION

This Chapter is designed to integrate functionally the individual components of the Masticatory System, as up to now only the anatomy of these components has been discussed.

Fig. 3.1 RELATIONSHIP BETWEEN JOINT, MUSCLES, AND TEETH




At the anterior end of the line are the teeth, which must contact their opponents in the opposite jaw to be of use, i.e. they must be in occlusion before they can function properly. In the Rat the dentition consists of two incisors and six molars in each jaw. The incisors are used in biting and gnawing, and are provided with sharp enamel edges. The molar teeth are used for grinding and

chewing food, and because the rat uses antero-posterior movements for chewing, these teeth have flat occlusal surfaces resulting from attrition occurring in the horizontal plane.

At the posterior end of the line is the temporo-mandibular joint. It is not only designed to make incision and chewing possible, but also for it to be carried out smoothly. To achieve this, the mandible (through the condyle) fits comfortably into the temporal articular site; it can rotate up and down to allow for opening and closure of the mouth and therefore incision, and can slide forwards and backwards without impediment within the temporal "gutter" to allow antero-posterior movements for grinding of food by the molars. The articular meniscus compensates for any discrepancies in form between the two articular surfaces during anteroposterior movements of the condyle. Because the mandible travels extensively anteriorly and hence produces great discrepancies for the meniscus to compensate for, the protrusion of the meniscus is controlled by an active protractor --- part of the lateral pterygoid muscle.

The active component of the masticatory system is the one in the middle of the line, the masticatory musculature. This unit moves the mandible on the temporal site, and controls it so that the teeth may be brought together and moved on one another, establishing efficiently the relationships necessary for masticatory function. It has been shown already (p.2.17) that for each acquired movement of the mandible, there is a specific contraction pattern of the musculature, and all this is controlled by the central nervous system on a feedback mechanism. When

relating masticatory function in the rat to muscular contraction, we have the following relationships:

(1) Opening before incision: the Anterior belly of the digastric is coupled with the action of lateral pterygoid;

(2) Closure as in incision, and in keeping the teeth together during grinding of food: Temporalis, masseter, medial pterygoid, acting together;

(3) Grinding of food: (a) Anterior movements: masseter, medial pterygoid, lateral pterygoid; (b) Posterior movements: anterior belly of digastric, deep head of masseter; (c) Keeping the teeth in contact: temporalis, part of medial pterygoid, and masseter.

One may summarise the functional occlusion of the rat by the following equations: (" + " = harmonious with)

(a) Temporal site + Meniscus + Condyle
= Harmonious temporomandibular joint.

(b) T.M.Joint + Muscles + Teeth
= Harmonious functional occlusion.

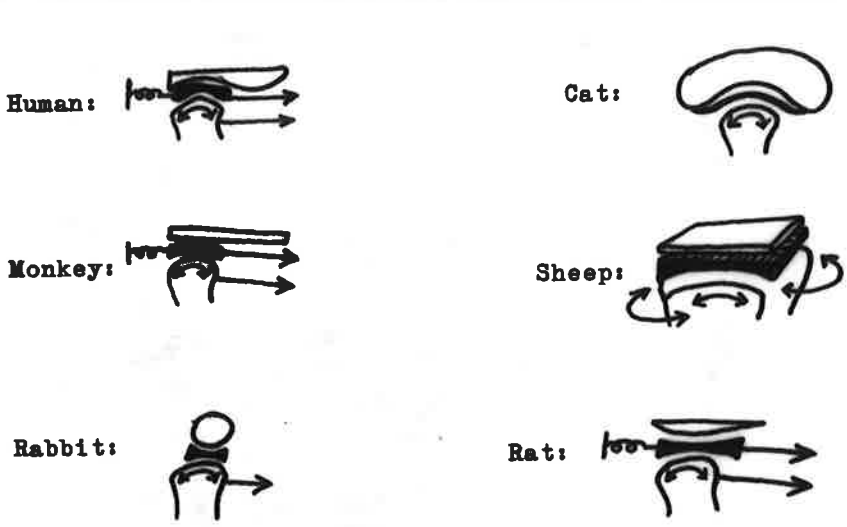
# CHAPTER 4

## COMPARATIVE ASPECTS OF THE RAT TEMPOROMANDIBULAR JOINT

Comparing the functional anatomy of the Rat's temporomandibular joint with those of other animals will enable us to comprehend better the subject of functional occlusion in the rat.

Detailed individual descriptions of the temporomandibular joints of Human, Monkey, Rabbit, Cat and Sheep are found in "The Temporomandibular Joint: A Comparative Study" (Lam 1971). A diagrammatic summary of the mechanics of the joints of these animals is given as follows:

Fig. 4.1 KINETIC MECHANISMS OF T.M.JOINTS of Human, monkey, rabbit, cat, sheep and rat:



It will be noted that the temporomandibular joint of the rat differs from the others in the following ways:

(1) With the exception of the rabbit, the rat's temporal site is the only one which is convex;

(2) With the exception of the Human and the Monkey which are Primates, only the rat has active protraction and retraction of the meniscus;

(3) The rabbit and the rat are the only animals under discussion where condylar lateral rotation or lateral play within the joint is not possible;

(4) The rat temporomandibular joint has no capsule forming part of the joint --- lateral rotation or splay is not required in mastication, and is prevented by the condyle fitting snugly into the temporal gutter. This "guttering" is so effective that a fibrous capsule designed to limit lateral movements is unnecessary. By contrast, the Sheep does require broad lateral rotation, and these movements are allowed for by a loose joint capsule. In the Human and the Monkey, such lateral rotation is not required, and is limited by the fibrous capsule and the medial and lateral ligaments of the joint. In the Cat, lateral rotation is not necessary and is prevented by the non-parallel hinge arrangement of the two condyles in the coronal plane; however some splay is possible in the cat, being necessary for the tight scissors action between the teeth, which is controlled by the two pterygoids which run in a medio-lateral direction.

Of the animals discussed above, the rabbit joint is closest in morphology to that of the rat. Because the rabbit and the rat have skulls which appear superficially to be alike, and both belong to the same Cohort Glires in

the Animal Kingdom, one is easily misled into thinking that their temporomandibular joints are similar. However, the existence of an active protractor mechanism of the meniscus (the lateral pterygoid having two insertions) in the rat provides a subtle difference. This we shall discuss after examining the muscles of mastication and the lateral pterygoid muscle itself.

Among the above animals, the rat has relatively a very large masseter which is horizontally placed, providing a strong anteriorly directed action which is necessary for anteroposterior movements during chewing of food. The upward action of the masseter keeps the teeth together during such movements, and its large size is associated with this major movement of the mandible during mastication.

The temporalis is stout and short and is designed for strength rather than for extensive movement. This is because in the rat elevation of the mandible is not extensive, but strong force is required to keep the teeth together during chewing and to provide at the same time effective vertical stabilisation.

The medial pterygoid of the rat is considerably smaller than the masseter. This may be due to the fact that both the usual actions of the medial pterygoid (forward and upward actions) are superceded by the two stout movers, the masseter and temporalis respectively. In the other animals where the medial pterygoids are also synergists with the masseter, they are quite comparable in size to the masseters. In the cat the medial pterygoid is much smaller than the masseter but this is understandable because the medial pterygoid

in the cat serves an entirely different function to that of the masseter, i.e. a lateral stabiliser rather than a protractor and elevator.

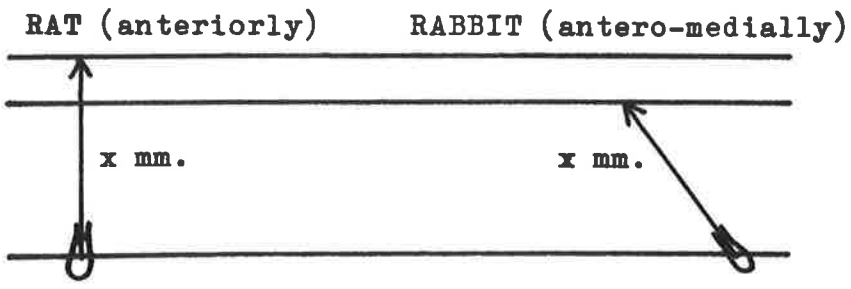
The lateral pterygoid of the rat is interesting as, like the other animals studied (except the Primates) it has only one origin, but unlike the other animals it has two insertions like the Primates: one into the mandibular condyle, and one into the articular meniscus of the Joint. Thus it not only protracts the mandibular condyle but also the meniscus. The absence of this meniscal protractor mechanism in the rabbit, cat and sheep, and its evolution in the Monkey and the Human is discussed in detail by the author (Lam 1971). Its existence in the rat provides the subtle difference between it and the rabbit, which superficially look similar. Also, the fact that it has only one origin (in contrast to two in the Human) places the rat Joint in an intermediate position between the Human and the Rabbit. This intermediate situation of the Rat's Joint can be understood by examining the shape of the articular surfaces and the movements relative to them:

Compared to the Human, the Rat's temporal surface is a simple convexity. The Human temporal site is concavo-convex and is distinctly more complex. Moreover, in the Human Joint the meniscus and the condyle move at different speeds (Rees 1954, Christensen 1969); the movements of the meniscus therefore require a precisely controlled protractor mechanism. This results in two separate components of the lateral pterygoid muscle (Protrudens menisci and lateral pterygoid proper of Christensen). In the Rat, control of protrusion of the meniscus is also necessary because of

extensive mandibular anterior movements (Young 1962, also p.2.20 of this Thesis), but because the temporal site is simply convex, control of the meniscus is not required to be as precise as in the Human. The rat's meniscus does not need to be moved at different speeds from that of the condyle in order to exert its compensatory effect. Therefore there is an active protractor but it has not evolved into having two separate origins. As in the Human, the active meniscal retractor is provided by the elastic fibres at the posterior extremities of the Posterior Bilaminar Zone of the meniscus (p.1.27 and p.1.28). These elastic fibres retract the meniscus as the lateral pterygoid relaxes during mandibular closure and retrusion.

Compared to the Rabbit, the temporal articular site is much longer and flatter. This is understandable, for, when the condyle travels a long distance anteriorly only, the anterior displacement is necessarily more than when it travelled through the same distance but anteromedially (see in the rabbit, Fig. 4.2). Furthermore, while the anteroposterior movements of the rabbit condyle may be seen as a relative "jiggle" on the temporal site, those of the rat condyle are extensive and definite. In fact when the lower incisors are brought into occlusion with the upper incisors, the mandible is so far forward that the molars are out of occlusion (p.2.20-2.21). Such long excursions would make the condyle drag on the meniscus, and it is to prevent this from happening that an active meniscal protractor has been evolved. This mechanism is not found in the rabbit. Active retractors of the meniscus (elastic fibres) are not found in the rabbit because no active retraction is needed.

Fig. 4.2 CONDYLAR PATHS of Rat and Rabbit




As for the overall size of the lateral pterygoid muscle of the rat, it is comparable in size to the same muscle in the other animals described above. Apart from protracting the meniscus, it protracts the mandible, and it also couples with the anterior belly of the digastric muscle in opening the mouth. In this respect it has a similar function to that in the other animals (with the exception of the cat as discussed already, c.f. lateral stabilisation by its pterygoids).

The anterior belly of the digastric in the rat is comparable in size to the digastric muscles in other animals. However, it is more horizontally placed, and this provides the strong and important posterior pull needed in mastication by the rat. Its slight downward incline is used to advantage in coupling its action with the lateral pterygoid in mandibular opening.

# SECTION II

# THE EFFECTS OF LOSS OF POSTERIOR OCCLUSION ON JOINT STRUCTURE

# CHAPTER 5

## PLANNING OF EXPERIMENTAL SERIES " E "

In this series of experiments all maxillary molars were removed from the rats under general anaesthesia. The methods used are discussed in Appendices II and III (p.A.3 to A.9).

While the rats were still able to incise and continue to take solid food with the teeth remaining in their jaws, the absence of all maxillary molars meant that the incisors would have to take up the total responsibility for mastication, and there would consequently be an increase in the rate of attrition. As the incisors of the rat wear rapidly, it was anticipated that this would decrease the occlusal vertical dimension of the face, i.e. the mandibles would be "overclosed". This experimental series was designed to examine whether this induced mandibular over-closure would bring about any detectable pathological changes within the temporomandibular joint at the end of various time intervals.

Ten Sprague Dawley rats were taken, from which all maxillary molars were extracted. At various time intervals from the day of extraction, rats were sacrificed for histological examination of possible changes in the temporomandibular joints.

The time intervals of sacrifice were as follows:

| Time | No. of Rats | Laboratory designation |
|---|---|---|
| 2 days | 2 | E |
| 1 week | 2 | E1 |
| 4 weeks | 2 | E4 |
| 8 weeks | 2 | E8 |
| 12 weeks | 2 | E12 |

Control of this series was established by using normal rats sacrificed at time intervals as described in Appendix I.

At least one joint from each experimental rat was examined histologically. Some were sectioned sagittally, some coronally.

Photomicrographs from each series of sections of the joints were then taken. These were carefully compared with the pictures of the normal rat so that any differences in histological appearances could be determined.

# CHAPTER 6

## RESULTS OF EXPERIMENTAL SERIES " E "

### TEMPORAL SITE

The following parameters were used in the assessment of results: (A) Under Low Power (x25): 1. the thickness of the fibrous covering, 2. the distribution of fibrocytes, 3. general structure of bone, and 4. the size of the marrow spaces. (B) Under High Power (x250): 1. the cells in the marrow spaces, 2. the size and shape of osteocytes, chondrocytes and fibrocytes, and 3. the structure of collagen bundles in the fibrous covering (their overall density and orientation).

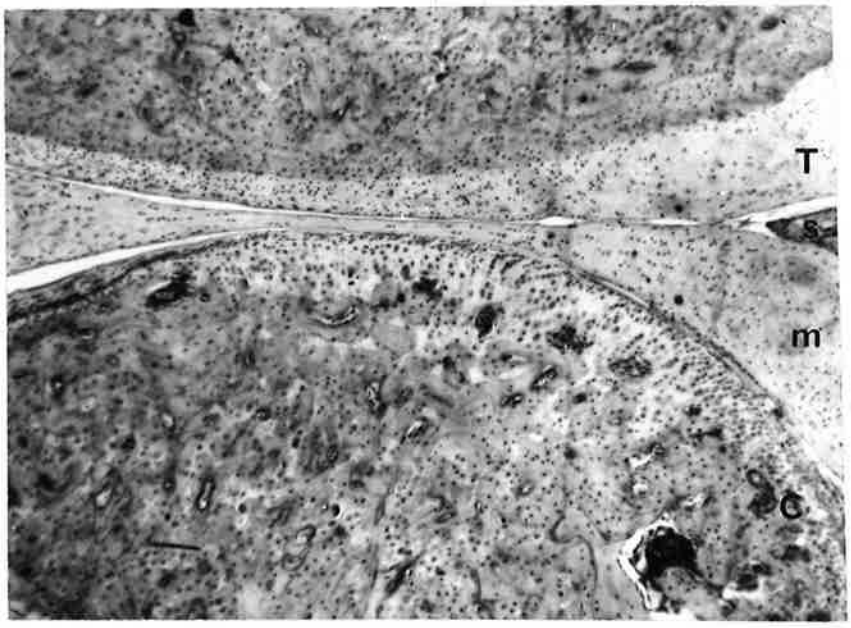
When compared with the Control series, it was observed that the structure of the temporal site in this series of rats (without posterior occlusion) was unchanged during the experimental period of 12 weeks.

Sections in the sagittal plane of this series of temporomandibular joints are shown in Figs. 6.1 and 6.2 (p.6.2).

Coronal sections of this series are shown in Figs. 6.3 and 6.4 (p.6.3).

Discrete areas of calcification and necrosis of cartilage as reported by Cimasoni (1963) and "osteosclerosis" as reported by Furstman (1965) are not seen in this study.

Fig. 6.1 SERIES "E", sagittal section, 12 weeks after extractions. H & E x25




T = Temporal Site

S = Synovium

M = Meniscus

C = Condyle

Fig. 6.2 SERIES "E", sagittal section, 12 weeks after extractions. H & E x250

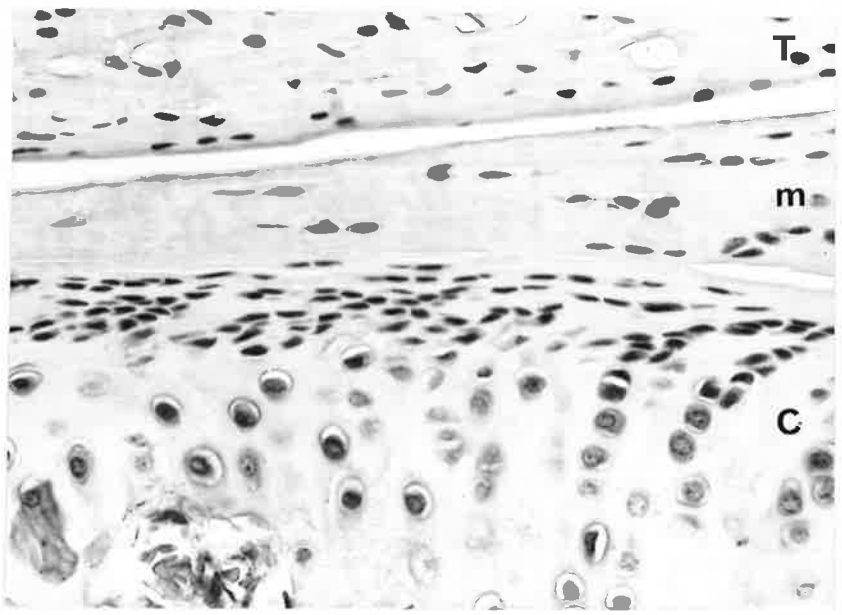
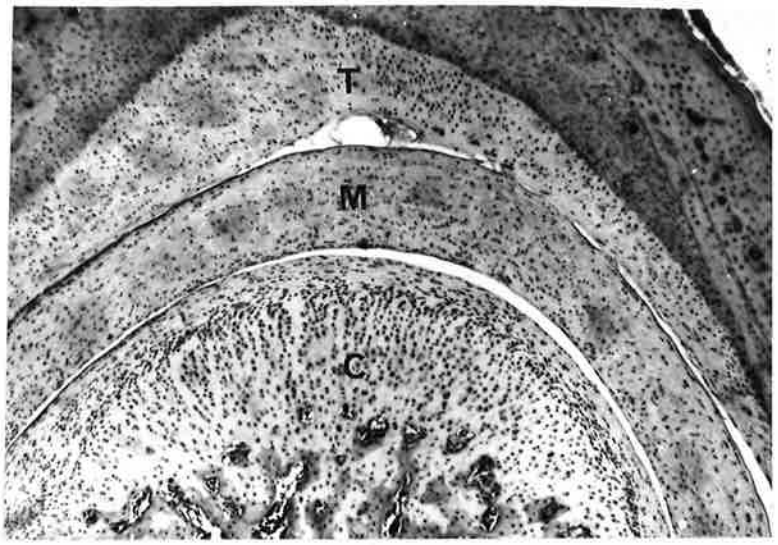


Fig. 6.3 SERIES "E", coronal section, 12 weeks after extractions. H & E x25

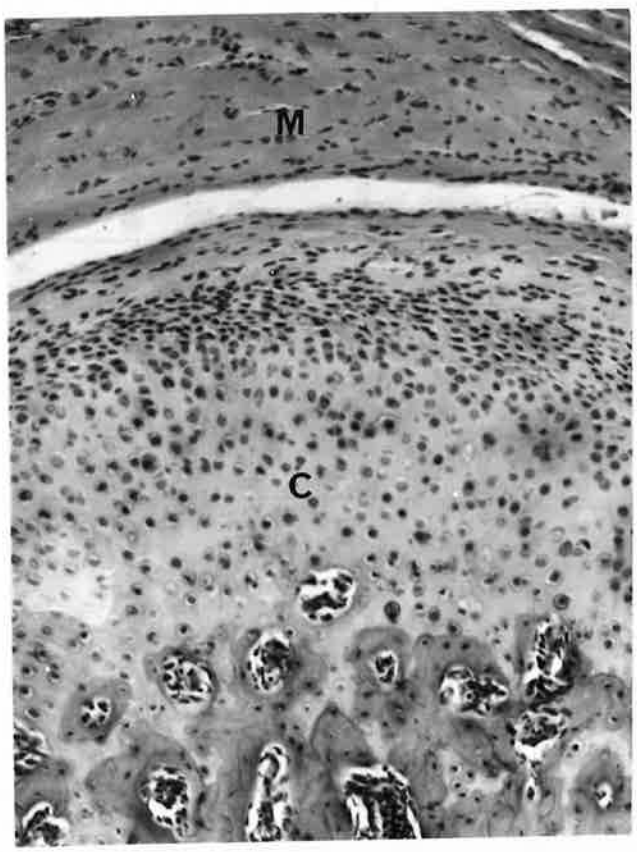



T = Temporal Site

M = Meniscus

C = Condyle

Fig. 6.4 SERIES "E", coronal section, 12 weeks after extractions. H & E x 100




M = Meniscus

C = Condyle

MENISCUS

The following parameters were used in the assessment of results: (A) Under Low Power: 1. the shape and thickness of the meniscus, and 2. the distribution of cells within it. (B) Under High Power: 1. the size and shape of the fibrocytes, and 2. the density and orientation of collagen bundles.

No alteration was observed in meniscal structure over the experimental period (12 weeks). The "isolated alterations" in meniscal structure (Cimasoni 1963) and the increase in meniscal thickness (Furstman 1965) were not seen over this period of time.

Sagittal and coronal sections are shown in Figs. 6.1 to 6.4.

CONDYLE

(A) Under Low Power the following were examined: 1. the thickness of the fibrous covering, 2. the thickness of the underlying cartilage, 3. the arrangement of the fibrocytes and chondrocytes, 4. the structure of bone, and 5. the size of the marrow spaces. (B) Under High Power: 1. the size and shape of fibrocytes, chondrocytes, osteocytes and marrow cells, and 2. the collagen bundles (density and orientation).

No change has been observed in this series. The reported "decrease in cartilage thickness" and "osteosclerosis" (Furstman 1965) have not been observed in either sagittal or coronal sections.

The results are given in Figs. 6.1 to 6.4.

## VASCULAR SYNOVIAL FOLDS IN THE UPPER JOINT CAVITY

Infolding of the retromeniscal pad of vascular synovial tissue and connective tissue was observed in normal and experimental rat joints in the upper joint cavity. This fold was usually seen to extend as far anteriorly as the posterior limit of the Posterior Thick Zone of the meniscus. Occasionally it may extend to the anterior part of the Posterior Thick Zone, but never beyond it (Figs. 1.11 & 6.1). From the results of this study, the inference is made that this infolding of the vascular synovial tissue has been erroneously interpreted as being a vascular "pannus" by Cimasoni (1963) (c.f. p.7.4-7.5).

# CHAPTER 7

## DISCUSSION : SERIES " E "

In view of the apparent absence of changes in the joint structures in this series over 12 weeks, a result which contrasts with reported alterations by previous authors (Cimasoni 1963, Furstman 1965), a critical examination of the findings must be made. A convenient approach to the problem would be to consider the findings under the following headings:

(1) Is the duration of the present investigation too short for structural changes to become apparent?

(2) Temporal site: Is the reported presence of "discrete calcifications, cartilage necrosis, and osteosclerosis" by the previous investigators a valid finding?

(3) Meniscus: Is the reported "isolated alteration in structure and increase in thickness" a valid finding?

(4) Condyle: Is the "decrease in cartilage thickness" and so called "osteosclerosis" a valid finding?

(5) Is the presence of a "vascular pannus" in the upper joint cavity a valid finding?

### (1) DURATION OF EXPERIMENTS

Furstman (1965) made his examinations at intervals of 2, 4, and 6 months respectively, and noted changes within the joint at 2 months. On this basis our present series which ran for 3 months seems to be of sufficient duration to

make it possible to compare results. Cimasoni's series (1963) covered a period of 300 days (10 months) but he noted histological changes only from 270 days (9 months) onwards. On this basis our present series is too short to observe the noticeable changes reported by Cimasoni. In view of these facts one needs to examine the changes reported by these authors critically.

## (2) VALIDITY OF TEMPORAL CHANGES

The "osteosclerosis" picture of Furstman (1965) does not appear convincing as there is an overall heaviness throughout the whole section suggesting overstaining. In fact it is difficult to understand why "sclerosis" should occur with mandibular overclosure. Even if it does, it is difficult to understand why sclerosis should be present throughout the whole section which includes areas of the temporal bone with which the mandibular condyle does not articulate. The sections shown were very heavily stained, and it is not possible to be certain that this indicated true "osteosclerosis".

As for the discrete perichondral calcifications and cartilage necrosis reported by Cimasoni (1963), it is not possible to compare the results of the present study with those of his study because his series ran for 2-3 times longer. However, the changes reported appeared minimal, and as the distribution of fibrocytes and chondrocytes, and the structure of collagen bundles and cartilage is not absolutely regular even in the normal joint, any observations under high power on "isolated derangements" in fibrous and cartilage tissues must be interpreted with reservation.

## (3) VALIDITY OF MENISCAL CHANGES

The reported "isolated structural changes" in the articular meniscus (Cimasoni) are again viewed with reservation for the reasons put forward in the paragraph above.

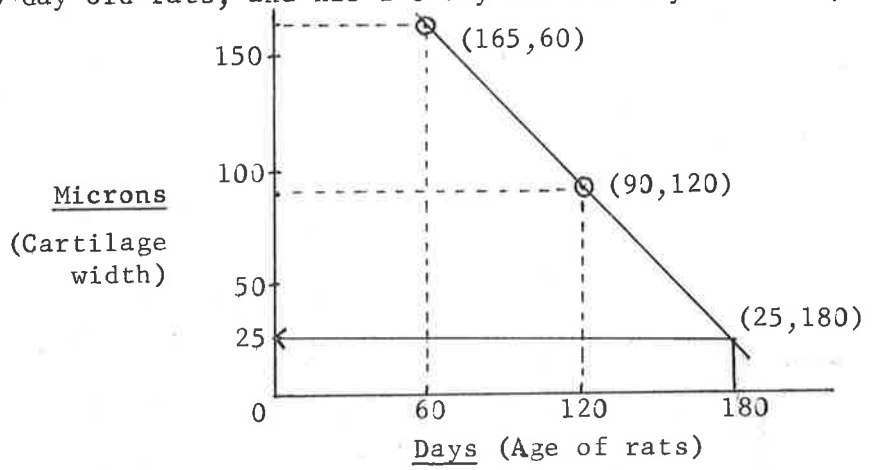
As for the "thickening" of the meniscus reported by Furstman, this was not seen in the present study with either sagittal or coronal sections. Coronal sections as used by Furstman cannot be relied upon to gauge the thickness of the meniscus because the meniscus has thick and thin zones in the antero-posterior direction (p. 1.25 to 1.27), and if coronal sections are made through the Intermediate Zone they will appear thin, while if made through the Posterior or Anterior Thick Zones they will appear thick. Furstman's pictures did not show thickening to any degree beyond the normal thickness of the Anterior or Posterior Thick Zones of the normal meniscus.

## (4) VALIDITY OF CONDYLAR CHANGES

The two condylar changes reported by Furstman are: (a) osteosclerosis, and (b) decrease of the cartilage cap width. Both of these findings meet with objections when viewed critically. First, these changes were supposed to occur from 2 months post-operatively, but were neither observed by Cimasoni (over 10 months), nor observed in the present study (over 3 months). Secondly, that the so called "osteosclerosis" was not convincing is pointed out above under the section on Temporal Changes (p. 7.2).

Thirdly, as for the decrease of cartilage width, Furstman described a decrease from 165 microns in 60-day old

rats and 90 microns in 120-day old rats to a "pathological" 25-45 microns six months after extractions of posterior teeth (i.e. 180-day old rats, or older). However, if one plots the two normal cartilage thicknesses against Age on a graph, one finds that even less than 25-45 microns would be normal for a 180-day old rat (assuming that the thinning of cartilage due to aging proceeds at the same rate between his 60-day and 120-day old rats, and his 120-day and 180-day old rats):




The conclusion is reached therefore that the "decrease in cartilage width" interpreted as a pathological phenomenon by Furstman was in fact not so.

## (5) VALIDITY OF THE VASCULAR PANNUS

Cimasoni described a "vascular pannus" in the upper joint cavity in experimental rats. In the present investigation an infolding of vascular synovial tissue in the upper joint cavity has been found both in experimental rats and in normal rats. Sagittal sections in this study demonstrated that this fold was limited to the posterior region of the joint only. It usually stretched no further

anteriorly than the posterior part of the Posterior Thick Zone of the meniscus, and never beyond the anterior limit of this thick Zone.

Cimasoni used coronal sections only in his investigations and it is obvious from present observations that sections through the anterior or middle part of the joint would not show this so called pannus, whereas those through the most posterior part would show it. In his paper, all the photomicrographs showing the "pannus" were associated with a thick meniscus, indicating that the coronal sections were made through the posterior region of the joint, where a synovial fold would normally be found. Moreover, his illustrations did not reveal any evidence of chronic inflammation (which is the basis of a pannus). It is considered therefore that this so called "pannus" is really an extension of the loose vascular retromeniscal pad of synovial tissue (c.f. Fig. 1.11 on p.1.18).

To summarise, the rat's temporomandibular joint is much longer antero-posteriorly than it is wide medio-laterally; there are thus wide structural and morphological variations within the temporal surface, the meniscus, and the condyle in the whole antero-posterior extent. Consequently interpretations using coronal sections only would be extremely misleading. What is more, the changes reported by Cimasoni in his 270-day old rats were <u>not</u> observed by him in his 300-day old rats. If changes were obvious in 270 days, it is expected they would be present also by 300 days. The reported "changes" by the previous authors on this subject can, therefore, only be regarded as "doubtful"

after a critical examination and a careful consideration of the methods used to prepare the material on which they were based.

There remains the possibility, however, that the present series extending over a period of 3 months may be too short in duration for noticeable changes to have occurred. Perhaps, by weakening the connective tissues in the Joint (by using lathyrism) changes might occur earlier. But before changes which occurred using this method could be interpreted, the changes caused by lathyrism alone would have to be excluded. For this reason the investigations recorded in Sections III and IV of this thesis were carried out.

# SECTION III

## THE EFFECTS OF LATHYRISM ON JOINT STRUCTURE

# CHAPTER 8

## NORMAL COLLAGEN : ITS FORMATION AND MATURATION

The general aspects of the structure of connective tissue is described on p. 1.10-1.17. COLLAGEN fibres are present in all connective tissue and are observed under the light microscope as long wavy structures which are referred to as collagen bundles. The bundles run parallel to one another, tend to converge and diverge, but rarely branch. Adjacent bundles however may run in different directions. Each of these bundles is made up of many fibres, and each fibre is made up of many fibrils. The fibril consists of macromolecules of the protein collagen. Examined under the electron microscope, the fibrils exhibit a characteristic periodicity of 640A, and are each about 100A thick (Ross & Benditt 1961). The molecular structure and arrangements of the macromolecules of collagen will be discussed below.

### CHEMISTRY OF COLLAGEN

The exact composition of collagen varies from type to type, but the following account will provide a general description of its components:

Of the total weight, over 99.5°/₀ consists of amino acids, the remaining 0.3-0.5°/₀ being the hexose sugars glucose and galactose. Among the amino acids, glycine is the most abundant, occupying about one-third in terms of molecules. Other amino acids include proline, alanine, hydroxyproline, serine, threonine, hydroxylysine, lysine,

arginine, glutamic acid, aspartic acid, leucine, valine, isoleucine, histidine, phenylalanine, methionine, and tyrosine. Of note, is the fact that the proportion of imino acids is higher in collagen than in most other proteins, and proline and hydroxyproline together account for about two-ninths of the amino acids in collagen, and are the two characteristic amino acid constituents of collagen. These two amino acids, together with glycine and alanine, make up two-thirds of the amino acids in collagen, leaving only one-third made up by the other fourteen amino acids.

The hexose sugars glucose and galactose are linked to the molecule by covalent bonds. No less than 75°/₀ of the hexose of the soluble collagen is attached to hydroxylysine (Cunningham et al. 1967).

The overall isoelectric point of collagen is at pH 9.4.

## MACROMOLECULAR STRUCTURE OF COLLAGEN

Ramachandran and Kartha (1954, 1955) suggested the triple-helical structure of collagen. The three polypeptide chains of collagen are each wound around its own axis in a simple left-handed helix of pitch 9A, having three amino acid units per turn. The three chains are arranged equidistant from one another, having every third amino acid unit from each chain placed at the central axis of the macromolecule, the units in turn equidistant from one another. The distance of one unit from another in the central axis would represent the phasic difference between the chains,

and would therefore be 3A apart (since three chains and the pitch of each is 9A). Bearing this arrangement in mind, the structure of the collagen macromolecule is obtained by giving this some right-handed twists with a pitch of 28.6A (Fig. 8.1 on p.8.5).

The length of the collagen macromolecules along the fibre is of the order of 3000A, and the molecules are aligned in a special manner to form the 640A repeating units. Each molecule would therefore run through four periods. Cross-linking among the chains takes place from side to side, having a gap of about 300A (Hodge, Petruska & Bailey 1965).

## SYNTHESIS OF THE MACROMOLECULES

Investigations into the synthesis of collagen have been reported by Ross and Benditt (1966), and Revel and Hay (1963). Both parties used radioactive tritiated proline, with the assumption that the paths taken by these particles would represent the paths of the collagen formed. Both found that the tritiated proline particles went to the endoplasmic reticulum first, and from this the inference was made that some degree of synthesis occurs there. From the endoplasmic reticulum it went outside the fibroblast, either directly (Ross & Benditt), or into the Golgi apparatus first before going outside the cell, the time taken for the two stops being 20 minutes and 2½ hours respectively (Revel & Hay). It is difficult to say to what degree collagen is synthesised before extrusion from the interior of the cell, but at least it is possible that synthesis of the macromolecules could occur inside the cell because of the lapse of time taken along this diverse route. These macromolecules

Fig. 8.1 Macromolecular structure

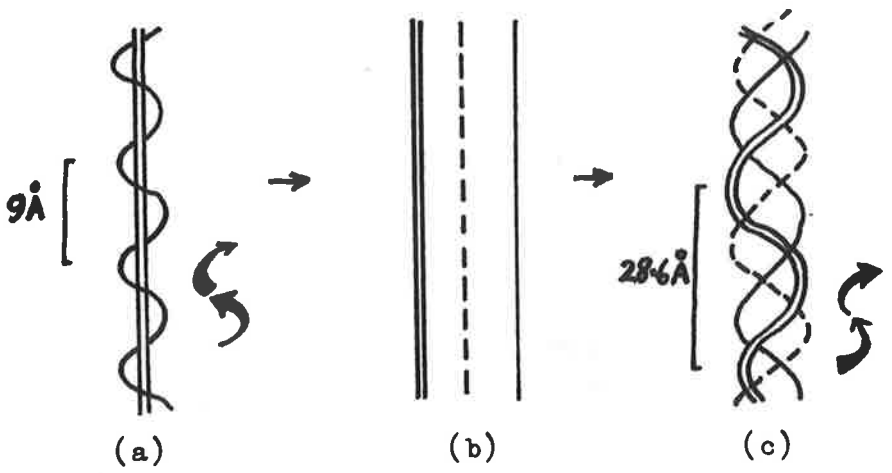
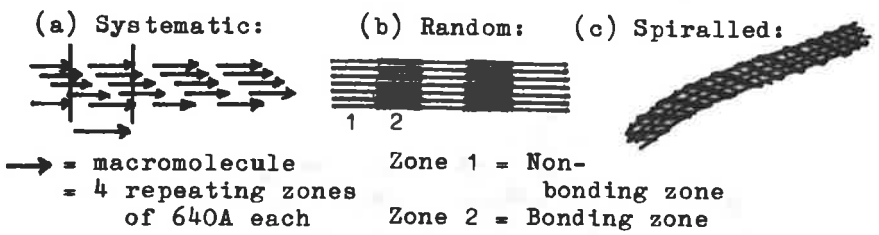



Fig. 8.2 Aggregation of Macromolecules



are then secreted extracellularly where they aggregate with others to form the collagen fibrils (Fitton Jackson 1964, 1967).

## FORMATION OF FIBRILS, AT MOLECULAR LEVEL

The regular 640A periodicity of a collagen fibril implies some kind of regular arrangement of the macromolecules, but the exact nature whereby this is produced is yet unknown. Furthermore, the orientation of molecules is dependent on the extracellular environment (Kent 1967). Fitton Jackson (1967) was of the opinion that this orientation does not take place within the fibroblast but in the extracellular environment, as a result of either enzymatic action at the cell surface, or a change of ionic environment or other such factors. Supporting this hypothesis is the possibility of fibril formation in vitro in a solution of soluble collagen, extracted from soft connective tissues, merely by suitably altering condition within the solution such as change in pH, salt concentration, or the presence of polyelectrolytes. This change presumably involves an electrostatic interaction between macromolecules resulting in their mutual orientation (Eastoe 1967).

Attempts at explaining the aggregation of macromolecules into the characteristic arrangement include the Systematic Aggregation Theory (Hodge et al. 1965), the Random Aggregation Theory (Grant et al. 1965), and the Spiralled Systematic Aggregation Theory (Veis et al. 1967) (Fig. 8.2 on p.8.5).

It should be pointed out that while analysis of

many collagens show that the molecules are essentially composed of the same amino acids, the pattern which the molecules form into different fibrils varies considerably. For example, the fibrils from the cornea have a smaller diameter than most collagens, being of the order of about 400A, and their banded pattern is very much less well-defined, this being highly characteristic of the cornea (Fitton Jackson 1967).

Formation of the collagen moiety goes hand in hand with the formation of the ground substance, which itself is characterised essentially by the presence of glycoproteins, acid glycosaminoglycans and non-collagen protein; these constituents of the ground substance may form specific three-dimensional arrays of multispecied macromolecular assemblies (Fitton Jackson 1965). Thus the ground substance may be a two-phase system in the sense that part may be directly associated with collagen and part may be organised as a three-dimensional coherent structure.

## FORMATION OF FIBRILS AND FIBRES, AT MICROSCOPIC LEVEL

Initial aggregation of collagen macromolecules occurs probably at the cell surface (Porter & Pappas 1959). The initial fibrils are too thin to exhibit the periodicity of 640A. Instead, they either have no periodicity or have a smaller one than the matured collagen fibres. These initial fibrils show no definite orientation, are very thin, argyrophilic, and arranged in a branched network. Later, numerous thicker wavy fibres are seen. These resemble mature collagen fibres but are still argyrophilic. Finally, thicker fibrils develop with the characteristic periodicity

of collagen and in bundles. Among the bundles, a network of branched argyrophilic fibres is still present (Gross 1961).

It should be noted that the individual increase in size of the collagen fibrils after their initial formation is due to the enlargement of each single fibril by the accretion of further molecules on to its surface and not by the aggregation of adjacent fibrils.

Stabilisation of the fibrils is due, in part, to cross-linking of the macromolecules by a process of enzymatic oxidation of lysine residues and, as the molecules become packed together, intra- and inter-molecular condensations of the aldehydes occur, resulting in a cross-linked insoluble fibril.

## MATURATION OF COLLAGEN FIBRES

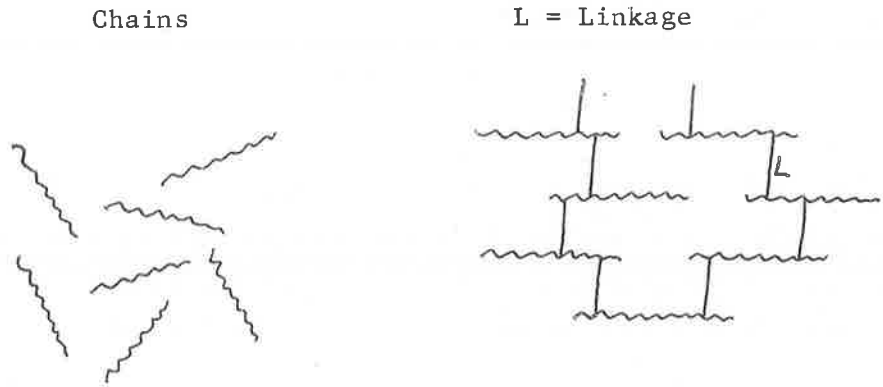
Newly-formed collagen fibres consisting of aggregated macromolecules are stabilised --- or matured --- by the formation of covalent cross-linkages both intra-molecularly between the chains in a single helix, and inter-molecularly between the chains in adjacent helices (Piez et al. 1961, 1963; Bornstein & Piez 1964, Lewis & Piez 1964, Courts 1961).

The introduction of covalent crosslinks during maturation does not necessarily proceed in a simple linear fashion, but both intra- and inter-molecular covalent bonding may proceed simultaneously and independently. The introduction of inter-molecular crosslinks appears to have a preference between alpha and alpha-1 chains, and between alpha-1 and alpha-2 chains of adjacent molecules. This

may be due to a particular steric relationship in the packing of the macromolecules in the fibrils of collagen due to specific interactions and compositional properties between the molecules (Veis & Anesey 1962, Glimcher & Krane 1968).

The process of collagen maturation appears to be a slow, spontaneous process, and progresses with time. The type of covalent cross-linkages present in mature collagen fibres have not yet been established with certainty. They may be ester links occurring in pairs and associated with hexose (Harding 1965). The nature of linking needs be surprisingly simple, involving only two linkages per chain, and all the fibrils are stabilised into a giant structure:

Fig. 8.3 Cross linkages in collagen fibrils



The above concept of collagen maturation is important for it is concerned in an understanding of Lathyrism (discussed in Chapter 9). Lathyrogenic agents such as amino-acetonitrile are responsible for the formation of weak connective tissue, seemingly acting by preventing formation of both intra- and inter-molecular crosslinks, possibly by blocking carbonyl groups.

## STAINING FOR COLLAGEN IN LIGHT MICROSCOPY

A few general stains reveal the bundles of collagen in connective tissues, but there is as yet no known histochemical stain that is specific for it. This is because the chemical groups in collagen are also present in the surrounding substances.

(1) Haematoxylin and Eosin: Haematoxylin stains collagen lightly, but is displaced when followed by eosin in the general staining methods.

(2) Mallory and Masson stains: These involve the use of amphoteric dyes such as aniline blue and light green which, together with phosphotungstic acid or phosphomolybdic acid reacting with amino groups and perhaps guanidino groups, demonstrate collagen well. However, these methods are not specific for collagen. Collagen is stained red.

(3) Van Gieson stain: This utilises acid fuchsin which reacts with hydroxy groups. This is relatively more specific for collagen as collagen contains a significant amount of hydroxyproline which is comparatively rare elsewhere. Collagen is stained red.

(4) Saturated Luxol fast blue G in methanol can be used to demonstrate collagen fibres (Salthouse 1965, 1966). Collagen is stained blue.

(5) Silver impregnation methods demonstrate collagen fibres by staining them golden brown.

(6) The use of antibodies for labelling collagen have been described by O'Dell (1965), Watson et al (1964), Rothbard & Watson (1962, 1965), Mancini et al (1965), Seifter (1965), Schmitt et al (1964), and Rubin et al (1965). A fluorescent material can be used to attach to the antibody thus showing it under the light microscope. For electron microscopy an electron-dense substance can be used. Antibodies to collagen and to a group of polar peptides obtained from the collagen macromolecule have been described by the above authors.

## CONNECTIVE TISSUE GROUND SUBSTANCE AND RELATIONSHIP WITH COLLAGEN

Connective tissue ground substances consist of two main groups: (1) Carbohydrate-protein compounds, and (2) Lipids. As only the former group is relevant in the present discussion it will be reviewed here.

The mucopolysaccharides of the ground substance are not free within the connective tissues but are linked to proteins. In joint spaces, for example, hyaluronic acid is associated with protein as a viscous complex of high molecular weight ($1 \times 10^7$ containing 25-30% protein) (Kent 1967). Similarly, chondroitin sulphate, keratin sulphates and heparin sulphates also exist in the same manner, attaching themselves to proteins by firm chemical bonds. The chondroitin 4- and 6-sulphates terminate in neutral sugar residues and the terminal residue is xylose which

is attached as a glycoside at the hydroxyl group of serine (Gregory, Laurent & Boden 1964, Anderson, Hoffman & Meyer 1965).

It is possible that in soft tissues the randomly coiled hyaluronic acid molecules enmesh proteins by a process likened to gel-filtration, behaving like a molecular net, whose interstices are penetrable by ions and small molecules but not large molecular substances. The behaviour of a filtering net may serve at least three purposes: (1) In the extracellular environment where flow occurs, the movement of high molecular weight substances would be favoured relative to small molecular weight substances; (2) By letting smaller molecules through and retaining the larger protein molecules, they concentrate proteins thus favouring deposition and fibre formation; and (3) The macromolecules of collagen are forced to channel between adjacent sheets of nets or through the larger holes in the nets themselves. This may have some influence in the directioning and orderly arrangement of forming collagen fibres.

It may therefore be said that the primary function of the carbohydrate-protein compounds in connective tissue ground substance is to provide an extracellular milieu which permits the subsequent formation and maturation of fibres as tissue development proceeds, and this function coincides with the histological concept of the ground substance, viz. in forming connective tissues of either a healing wound or embryonic structures, there is a proportionally smaller content of collagen fibres but a larger amount of carbohydrates. Another function of some of these compounds is to hold a very large volume of water among their net-like molecular form (chondroitin sulphate and hyaluronic acid), hence providing an efficient means of occupation and organisation of extracellular space (Melcher & Bowen 1969).

## Biochemistry of Carbohydrate-protein compounds

The amounts and types of mucosubstances vary from tissue to tissue within the same animal and, in each tissue, vary with the age of the animal. A basic typing may be made into (1) Polysaccharide-protein compounds; and (2) Glycoproteins.

(1) Polysaccharide-protein compounds: These include hyaluronic acid, chondroitin sulphates A B & C, chondroitin, heparin sulphate, and keratosulpate. They are characterised by highly polymerised carbohydrate chains containing up to several thousand units of sugar and having a regular repeating sequence, usually with two kinds of sugar units alternating along the length of the chain. One of these two units is usually a hexosamine, and the other a hexuronic acid. Polysaccharides represent the major part of these substances but are nearly always associated with a small amount of protein through covalent bonds. It is possible that the polysaccharides and protein together make up a very large molecule having perhaps many carbohydrate chains (Gottschalk 1966).

Hyaluronic acid, for example, contains 25-30% of protein probably loosely associated with the carbohydrate. The particle-weight is about $1 - 4 \times 10^6$. The structure consists of a very long polysaccharide chain which is not tightly rolled up because of mutual electrostatic repulsion between the negatively charged carboxyl groups on alternating glucuronic acid units. It is quite flexible because of being long and thin.

Chondroitin sulphate, as another example, has a

molecular weight of about 1 - 5 x $10^6$. The structure consists of a protein core bonded covalently with some 30-60 polysaccharide chains. Each of the polysaccharide chains is of molecular weight approximately 50,000. Unlike hyaluronic acid, chondroitin sulphate molecules are rigid because of mutual repulsion of negatively charged groups on the carbohydrate chains. The length of the molecule is 3700A (Muir 1964, Partridge et al. 1961).

As maturation of connective tissue proceeds, the hyaluronic acid content decreases and chondroitin sulphate increases. The changes in the biosynthesis and biochemical pattern of the ground substance is associated with the laying down of collagen fibres. Also, conditions leading to the impairment of formation of mucopolysaccharides will lead to defective connective tissues, e.g. scurvy.

(2) Glycoproteins: This is the other group of Carbohydrate-protein compounds. Unlike the polysaccharide-protein compounds, the glycoproteins are usually mainly proteins having a lower carbohydrate content. They are conjugated proteins having one or more heterosaccharides with a lower number (2-20) of sugar residues. The sugar units, again differing from the polysaccharide-protein compounds, lack a regularly repeating sequence. In composition, they are similar to the polysaccharide-protein compounds in having N-acetyl-D-glucosamine and N-acetyl-neuraminic acid (sialic acid) is characteristically present. Sialic acid has a strongly acidic carboxyl group (pK 2.6), and if present in sufficient amounts will give the whole glycoprotein molecule an acidic character.

Sialoprotein in bovine bone may be used to illustrate this group of compounds. It is a highly branched carbohydrate chain with sialic acid units in the terminal positions, joined through galactose, hexosamine and two other sugar units to a single glucosamine unit attached to the protein part of the molecule. Its molecular weight is about 23,500 (Andrews & Herring 1965).

The relationship of these carbohydrate-protein compounds in the ground substance to the formation of collagen fibres is important. In the regeneration and repair of tissues the process retraces the stages of formation of these compounds (i.e. hyaluronic acid to chondroitin sulphate to keratan sulphate) as accompaniments to collagen formation. Collagen deposition occurs at the height of metachromasia (Dunphy & Udupa 1955, Slack 1957, Fernando & Movak 1963). As collagen matures, metachromasia decreases, implying that these compounds either disappear or become incorporated into the fibres so that they are no longer demonstrable by metachromatic staining methods. Another relationship of these compounds with collagen formation is their net-like form which may contribute to the formation of collagen in the form of fibres as mentioned on p. 8.12. Finally, these compounds in the ground substance may be part of a two-phase system which could be directly associated with collagen deposition (Fitton Jackson 1965).

In histological techniques, these mucosubstances may be demonstrated by a number of methods, such as the Periodic acid Schiff reaction (McManus 1946), and the metachromatic stain (Schoenberg & Moore 1964).

## REMODELLING OF COLLAGEN

Connective tissues are dynamic structures and are capable of undergoing modification of their shape, structure or metabolism in response to disturbing factors or in accordance with regulating processes. The catabolic cells are the macrophages and histiocytes of soft connective tissues, the osteoclasts of bone and the chondroclasts of cartilage. Where abnormal stresses are placed on these tissues, one may find an increase in number of these cells and a modification of tissue form.

Microscopically, remodelling may be described in three stages: (1) the arrays of collagen fibres of the basement lamella are first dissociated by swelling, and are invaded by cellular processes; (2) the collagen fibres then break down, and cellular invasion increases; and (3) the fragmented collagen fibres are then phagocytosed, and new fibres are formed in bundles. Throughout, there is no disturbance in function of the tissue.

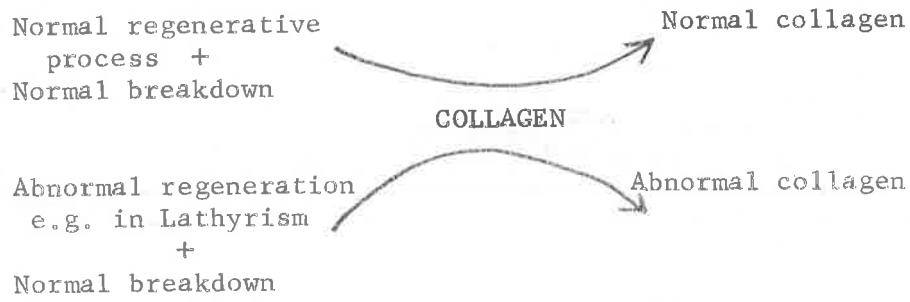
Native collagen is very difficult to degrade and a specific protease has never been extracted from animal tissues. The only collagenases able to break the peptide bonds of native collagen which have been known for some time are bacterial in origin and are therefore not pertinent to the present investigation. However, in tissue cultures a specific collagenase has been isolated, although not in vivo (Lapiere & Gross 1963).

Other enzymes like hydrolases and cathepsins in considerable variety appear to be stored in connective tissues, possibly in the lysosomes (Eeckhout 1964). Tissue resorption

is accompanied by an increase in these hydrolases. They are probably required for removing various tissue proteins and possibly degrade collagen further into peptides and amino acids after it had first been denatured by collagenase.

We can thus see that the collagen bundles in tissues are dynamic, and that the collagen observed under the light (and electron-) microscope represents only a cross-section of what was happening at the time of fixation. In the present study one is not concerned about the detailed biochemistry of the dynamics of collagen, but the concept of constant collagen breakdown and regeneration is important. This is illustrated by Fig. 8.4:

Fig. 8.4 Dynamic concept of collagen



# CHAPTER 9

## LATHYRISM : THEORY AND REVIEW

The term "Lathyrism" is used when referring to a syndrome produced by the ingestion of certain legumes of the family Lathyrus. As early as 500 B.C., Hippocrates noted that certain humans became diseased after the protracted consumption of some kind of peas. The species of the family Lathyrus which can cause lathyrism in humans are L. cicera, L. clymenum, and L. sativus. These produce damage to the brain and spinal cord, and the disease syndrome may therefore be called "neurolathyrism" (Selye 1957). These species of the family Lathyrus are, however, non-toxic to rats (Lewis et al. 1948).

In rats, a second type of lathyrism can be produced by the ingestion of L. odoratus (sweet peas), L. hirsutus, L. pusillus, or L. tingitanus. The active ingredient as an extract or synthetic derivative will, of course, produce the same effects. The syndrome consists of weakening of the skeletal system and other mesodermal tissues which are made up of connective tissue fibres, and it may be called "osteolathyrism" (Selye 1957). It is this type of lathyrism with which we are concerned in this study.

The active toxic ingredient in Lathyrus odoratus has been extracted and identified as beta-aminopropionitrile (BAPN), whose formula is $NH_2.CH_2.CH_2.CN$ (Dupuy & Lee 1954, McKay et al. 1954, Schilling 1954, Schilling & Strong 1954,

Bachhuber & Lalich 1955). Its related synthetic compounds are also effective, of which amino-acetonitrile is the most potent (Sarnat & Sciaky 1965).

## BIOCHEMISTRY OF LATHYRISM

Levene & Gross (1959) found that lathyrism enhanced collagen breakdown by producing a defect in the aggregation of the macromolecule to form collagen fibrils by preventing the formation of cross-linkages between the macromolecules during maturation (Maturation has been discussed on p. 8.8). The collagen molecules produced are only alpha-chains; the dimers beta-gelatins are not produced (Martin et al. 1963). Thus the newly synthesised collagen remains readily soluble, and the connective tissues are weak and easily disrupted by physical stress. Tendons, fibrous tissues and bones have less tensile strength than normal, and their structural integrity can be easily disrupted by a minimum of force (Levene & Gross 1959, Gross et al. 1960).

That lathyrism affects mainly newly-formed collagen has also been demonstrated by the use of tissue culture (Golub et al. 1968).

The exact mechanism of lathyrism is not known for certain, although one can feel sure that it does not disrupt the macromolecular structure itself. This is because both normal and lathyritic macromolecules have been found to be identical in shape, size, conformation and denaturation temperature. Also, lathyritic collagen molecules can be reconstituted to form native collagen fibrils (Levene & Gross 1959, Gross 1963). It is therefore most likely that

lathyrism interrupts the inter-molecular bonds in formed collagen during the maturation stage when soluble collagen is converted to insoluble collagen as a result of a closer and more perfect packing of macromolecules in the fibril with the exclusion of the solvent water. Lathyrism may act by preventing the formation of the stereo-specific short range inter-molecular forces. It may achieve this by changing the functional groups in the molecules or somehow preventing them from packing closely together and excluding water (Gross 1958, Levene et al. 1966, Tanzer et al. 1966).

Lathyrism also leads to an accumulation of muco-proteins (p.8.13 - 8.15) in the connective tissues. It has therefore been suggested that blockage from their further metabolism in the extracellular matrix may occur (Flieder & Schneider 1966). Whether this would disturb the interphase system between extracellular polysaccharide-protein compounds and collagen fibre formation causing a slowing down of its generation is not yet known.

## LATHYRISM IN ANIMALS AND TEMPOROMANDIBULAR JOINTS

Experimental lathyrism on various animals has been studied for many years and disorders have been noted to include retardation of growth (both pre- and post-natally), kyphoscoliosis, joint dislocations, deformations of long bones, dissecting aneurysms of the aorta and changes in the dental tissues and the mandible (Abramovich & Devoto 1968, Geiger et al. 1933, Ponseti & Baird 1952, Chang et al. 1955, Bachhuber & Lalich 1955). Research on lathyritic perio-dontal membranes, alveolar bone and epiphyseal cartilage of

long bones may give some insight into what one may expect to find in fibrous connective tissues, bone and cartilage in the body at large (Gardner et al. 1958, Gardner 1959, 1960, 1964, 1966, Ponseti & Shepard 1954, Krikos et al. 1958, Sciaky & Ungar 1961, Sarnat & Sciaky 1965, Ellen 1969). These observations include: (1) In the periodontal membrane: swelling of fibroblasts and their arrangement in palisades, slight increase in the number of mitoses, appearance of hyaline bodies of non-fibrous collagen --- these "lathyritic bodies" are often intensely eosinophilic and surrounded by the fibroblastic palisades, and disorientation of collagen fibres; (2) In the alveolar bone: Increase in osteoclastic activity, decrease in trabeculae and loss of orientation, and increase of marrow spaces with presence of loose connective tissues; (3) In cartilage: hydropic degeneration of chondrocytes, formation of microcysts, and invasion by capillaries; (4) Alveolar bone changes are accentuated but remediable to a great extent by the dietary supplementation of casein and gelatin (Gardner 1959); and (5) Changes in the dental tissues have been shown to be reversible by the return to a normal diet; this starts from the third to fifth day and becomes complete by the end of two weeks (Barrington & Meyer 1966).

Research directly applied to lathyritic temporomandibular joint structures has been limited. Krikos et al. (1958) reported exostoses on the mandible where tendons and ligaments were attached, a thickened and cellular periosteum with palisading of cells and little intercellular material, and disorientation of collagen fibres consisting of finer fibrils than normally found (viz. histological changes are similar to those reported for lathyritic connective tissues

in general). With condylar bone, they found increased osteoclastic activity, and in the exostoses eosinophilic material was present with many associated cells, these being replaced later by immature bone which was not compact. With condylar cartilage, they found it to be enlarged and thickened, more cellular, but with chondrocytes arranged haphazardly and uniform in appearance from the superficial to the deep layer (rather than showing a transition as in a normal condyle, see p.1.22). Sarnat & Sciaky (1965) reported similar cartilage changes. Gardner et al. (1958) and Gardner (1960) also reported exostoses on the mandible as described above; in addition, they reported deposition at the lip of the temporal bone thus deepening the "gutter". With connective tissues, they found proliferation of cylindrical or spindle cells which in some areas assumed palisade arrangements; later, hyalinisation occurred between these palisades and some cells appeared similar to osteoblasts. After 3 weeks, some bony trabeculae had developed. With bone, they found increased osteoclastic resorption resulting in irregular spongiosa and widening of marrow spaces, and the condyle after some weeks was distorted in form and consisted of only proliferating connective tissue and a few bony trabeculae. These findings are similar to those of Krikos et al. (1958). With the condylar cartilage, however, they noticed two results: in one group of rats there was continued interstitial proliferation of chondrocytes with calcification, there being no sign of degenerative changes; chondrocytes tended to segregate into small groups with much intercellular material between them, the normal orderly arrangement having been lost. In the other group there was thinning of the condylar cartilage with eventual disappearance. These findings differ from those of

Krikos et al. (1958). From the above reviews the following factors merit consideration:

(1) What are the long-term changes in the temporomandibular joint with lathyrism? None of the above experiments exceeded four weeks of feeding on a lathyrogenic diet.

(2) What is the final result with the condylar cartilage? Will it continue to proliferate and thicken, or will it disappear entirely?

(3) None of the articles mentioned changes, if any, in the articular meniscus. What changes may there be?

(4) What changes are there in the temporal articular site, and what alterations may there be with the layer of cartilage present there?

(5) It is difficult to show any changes in the anterior and posterior parts of the joint by using coronal sections alone, as were used by the above-mentioned authors. To demonstrate changes, serial coronal sectioning would need be carried out. This was either not done or due to lack of space was not reported by them.

In an attempt to find an answer to the above questions, in the present investigation sectioning in both sagittal and coronal planes has been carried out, the results of which are reported in Chapters 11 and 12.

# CHAPTER 10

## PLANNING OF EXPERIMENTAL SERIES " L "

In this series of experiments, 10 Sprague Dawley rats were placed on a lathyrogenic diet consisting of a 50/50 mixture of Lathyrus odoratus seeds and normal rat food pellets (see Appendix IV).

The various time intervals for sacrifice of the rats were as follows:

| Time | No. of rats | Laboratory designation |
|---|---|---|
| 2 days | 2 | L |
| 1 week | 2 | L1 |
| 4 weeks | 2 | L4 |
| 8 weeks | 2 | L8 |
| 12 weeks | 2 | L12 |

The method of using controls with normal rats is discussed in Appendix I.

This series of experiments was designed not to run for an extended period, as deaths were reported to occur at 4 to 5 months, sometimes even earlier (Sims 1972). The present study which extended over a period of 3 months was thus meant to allow for maximum structural changes without the risk of any rats dying prematurely.

For the same reason, the lathyrogenic sweet-pea seeds were mixed with normal rat food in order to prevent too severe an effect on the rats which might again lead to early deaths.

At least one joint from each rat was examined, some sectioned sagittally, some coronally. Photomicrographs were taken from each series of sections. These were carefully examined and compared with the photomicrographs of normal (control) rats to determine the incidence of pathological changes at the respective time intervals.

# CHAPTER 11

## RESULTS OF EXPERIMENTAL SERIES " L "

When examining sections of this series, the same parameters were used as in those of the "Extraction" series (Series "E") (Chapter 6, p.6.1 and p.6.4).

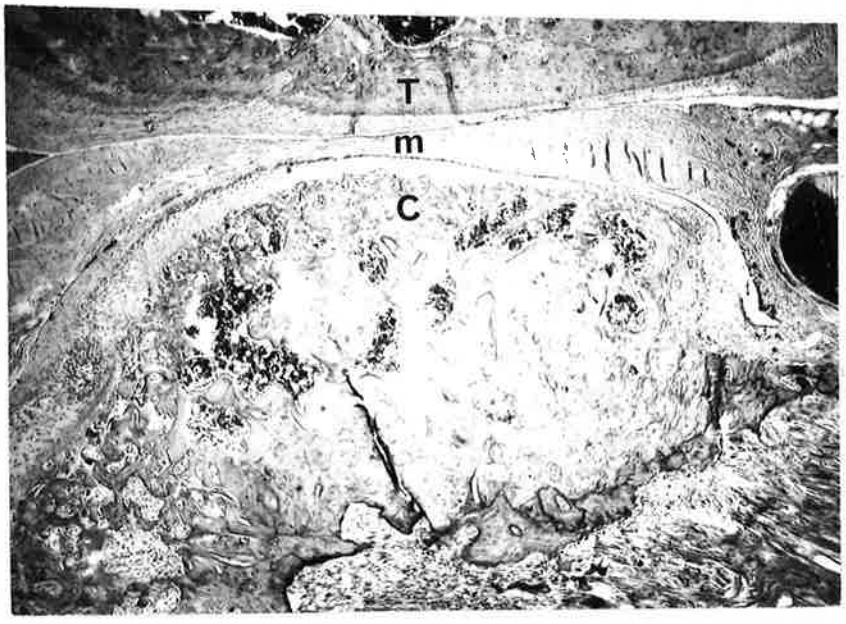
### TEMPORAL SITE

Changes in the temporal site were variable. Most rats did not show any change, but in one rat the lateral wall of the temporal site was partly replaced by fibrous tissue (Fig. 11.5 on p.11.4), and the fibrous lateral wall in this case seemed thicker than the normal wall (compare with Fig. 1.12 & Fig. 6.3).

In this rat the change took place one week after commencing the lathyrogenic diet. Because the other rats did now show this change, the inconsistency of this finding could be due to an oblique orientation of the joint (i.e. not exactly coronally sectioned), giving a possibly misleading "thickening". In view of this, the thickening should be disregarded.

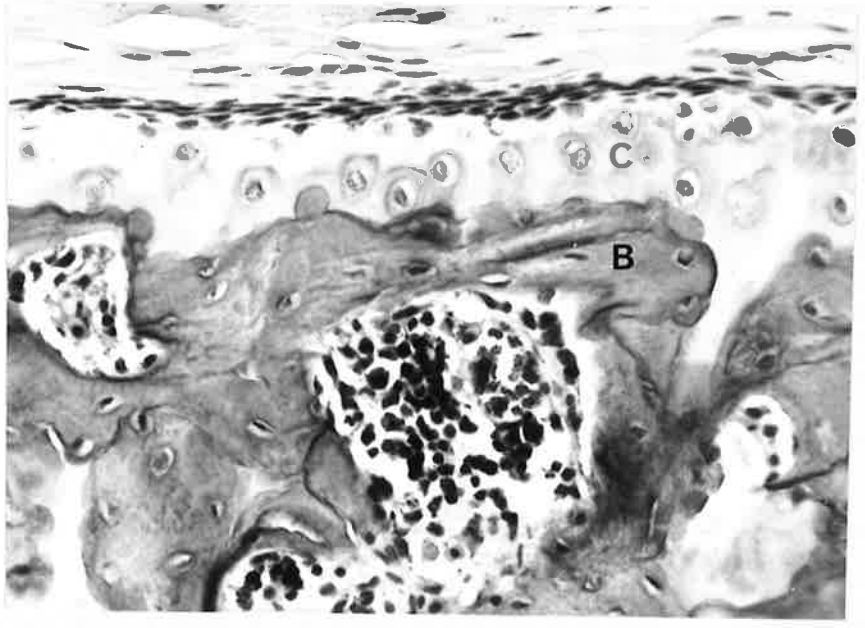
No thinning of bony trabeculae or enlargement of marrow spaces (such as observed in condylar bone) was noted.

Fig. 11.1 SERIES "L", sagittal section.
H & E x25




T = Temp. site
M = Meniscus
C = Condyle

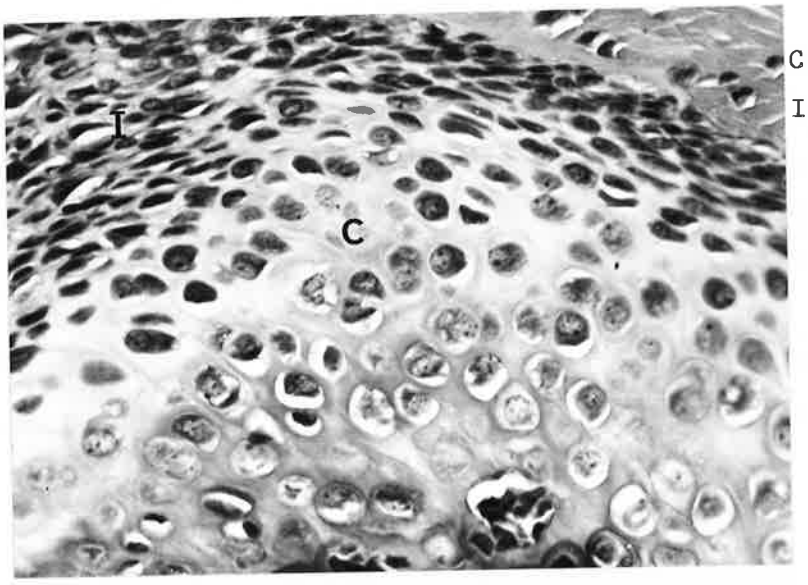
Fig. 11.2 SERIES "L", Thinned anterior condylar cartilage.
H & E x250




C = Cartilage
B = Bone

Fig. 11.3 SERIES "L", Thicker posterior condylar cartilage (adjacent to chondrocyte "island")

H & E x250

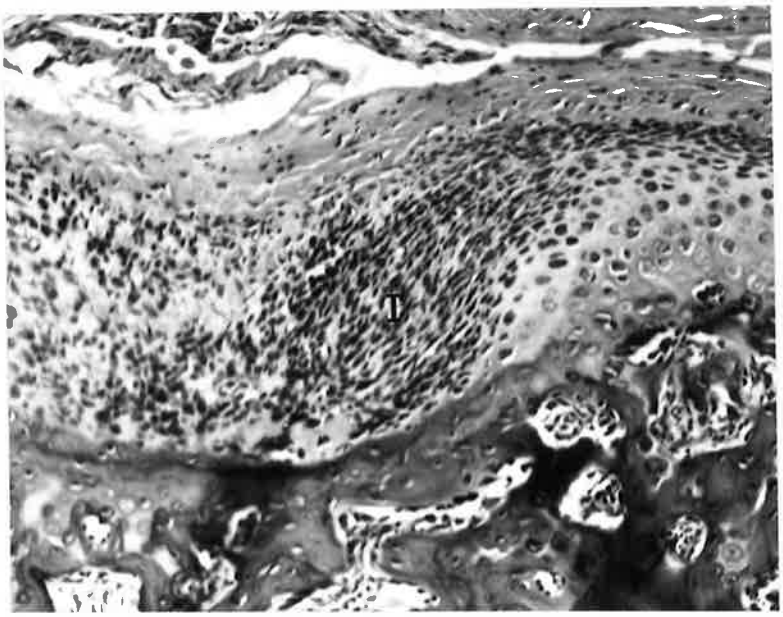



C = Cartilage

I = Island

Fig. 11.4 SERIES "L", Chondrocyte "island" in posterior condylar cartilage

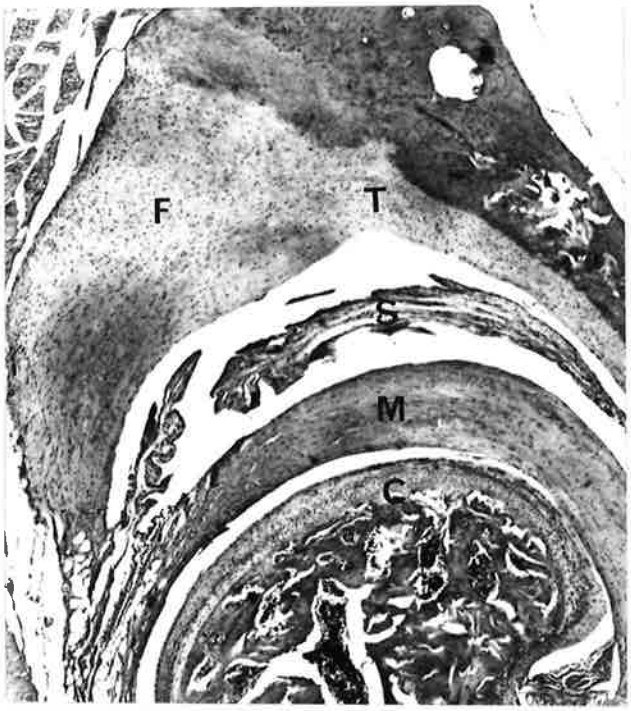
H & E x100




I = Island

Fig. 11.5 SERIES "L", coronal section

H & E x25




T = Temp. site

F = Fibrous thickening of lateral wall

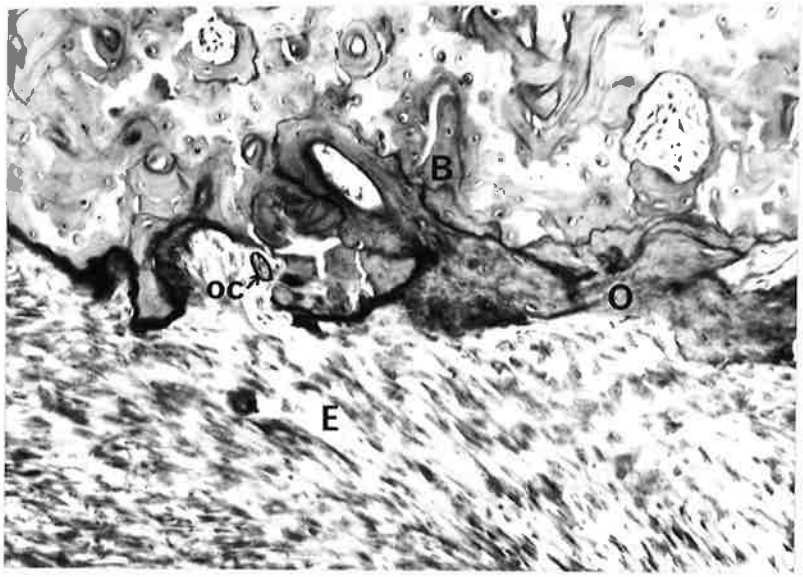
S = Synovium

M = Meniscus

C = Condyle

Fig. 11.6 SERIES "L", At 12 weeks, H & E x100

Osteoclastic resorption of condyle at lateral pterygoid attachment, and presence of osteoid.




B = Bone (with reversal lines)

O = Osteoid

E = "Exostosis"

OC = Osteoclast

MENISCUS

No noticeable change was observed in the meniscus with respect to size, shape, or structure, even after 12 weeks on the lathyrogenic diet (Figs. 11.1 to 11.4).

CONDYLE

Several changes have been observed in the lathyritic condyle: (Figs. 11.1 to 11.9)

(1) The most striking change is severe resorption of bone at the attachment of the lateral pterygoid muscle, and its replacement by massive amounts of cellular fibrous tissue, forming the "exostosis" described by previous authors (Chapter 9). Figs. 11.7 to 11.9 show a Van Gieson stain of such an area.

(2) The "exostosis" *(see footnote) altered the shape of the condyle. It consists of compact arrangements of long fibroblasts, streaks of immature collagen, and remnants of lateral pterygoid muscle fibres. The fibroblasts are orientated in the direction of muscle pull. At the junction of the "exostosis" with bone, there are many areas of bone resorption with osteoclasts present, and with no definable periosteum. There is immature bone (osteoid) and many reversal lines. Osteoid occurred from 4 weeks onwards after feeding on the lathyrogenic diet.

*N.B. The term "exostosis" has been used by previous authors on the subject, and is used here for this purpose. It is recognised, however, that the real meaning of the term does not apply to this non-bony hyperplasia of fibrous tissue.

Fig. 11.7 SERIES "L", Photomicrograph of FIBROUS EXOSTOSIS of mandibular condyle.

(Specimen at 12 weeks, Van Gieson Stain x25).

C = Condyle
FE = Fibrous exostosis

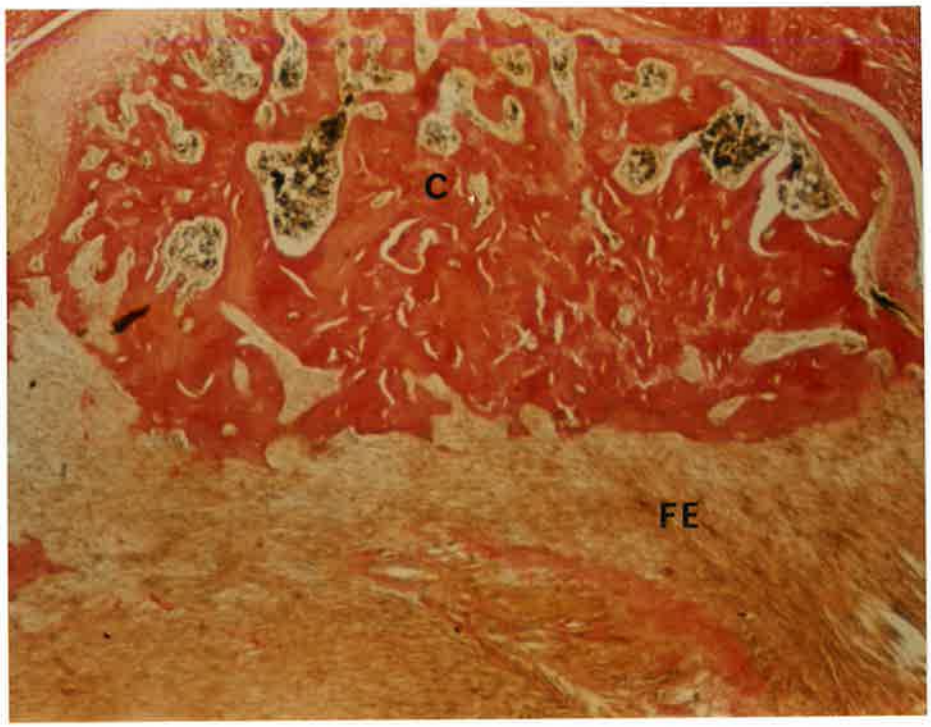


Fig. 11.8 Photomicrograph of Fibrous Exostosis, showing a region with mostly fibroblasts and very little mature collagen.

(Van Gieson x250)

F = Fibroblast
C = Collagen

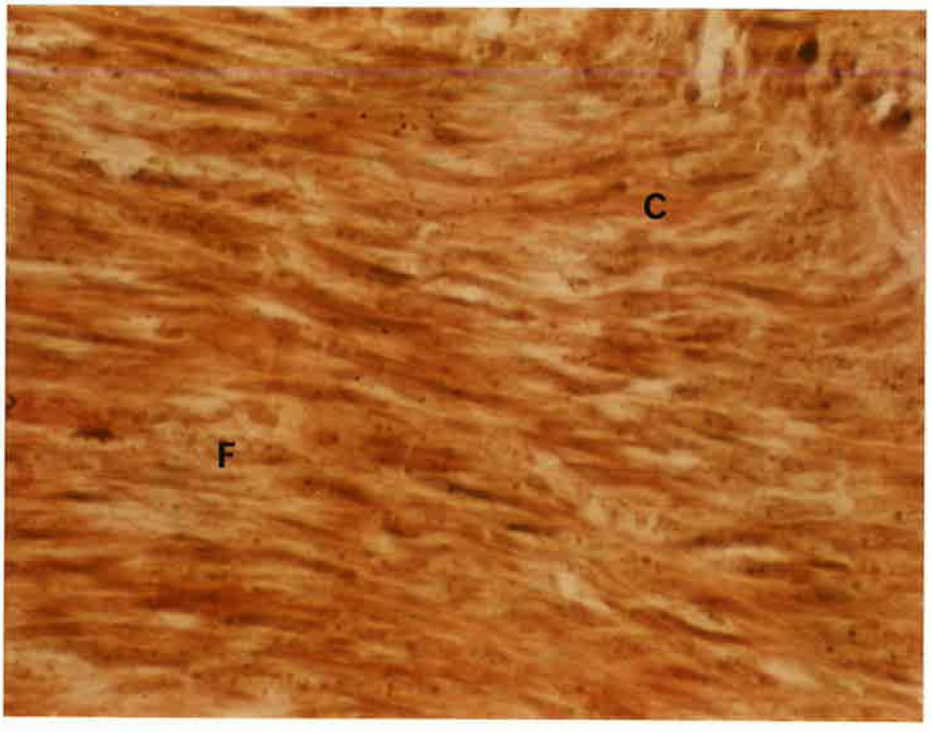
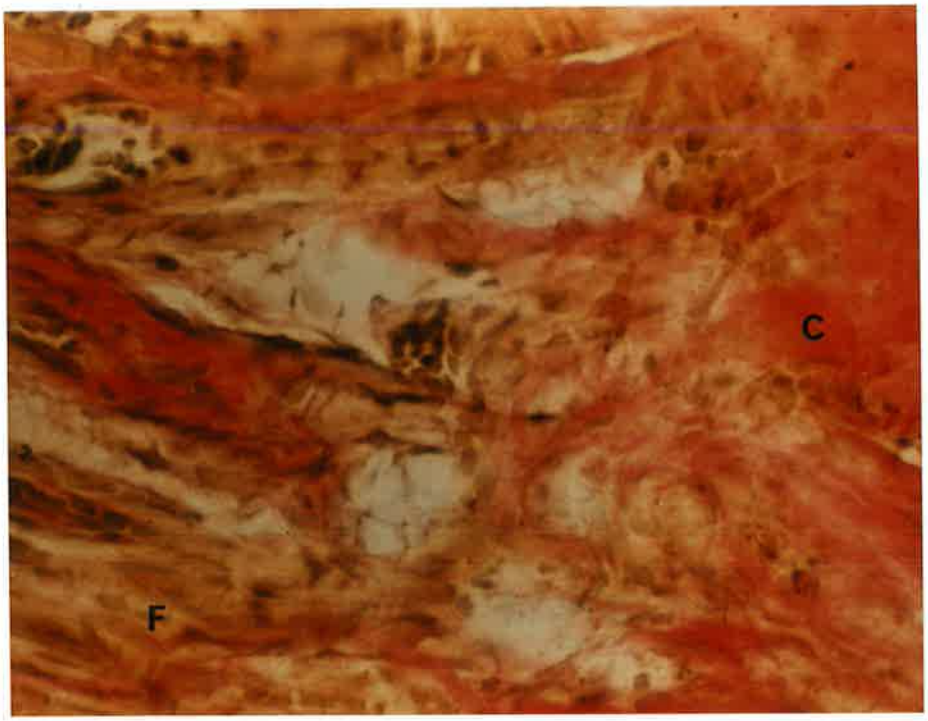


Fig. 11.9 Photomicrograph of Fibrous Exostosis, showing an area with relatively more demonstrable collagen among fibroblasts.

(Van Gieson x 250)

C = Collagen
F = Fibroblast



(3) Anteriorly, the fibrous layer of the covering of the condyle is thin, the oblique transitional layer of fibrocytes having been lost. A thin layer of condylar cartilage (about 2 cells thick) merges into bone. Coronal sections show an uneven distribution of fibrocytes, some of which are grouped into 2 or 3, some 4 or 5.

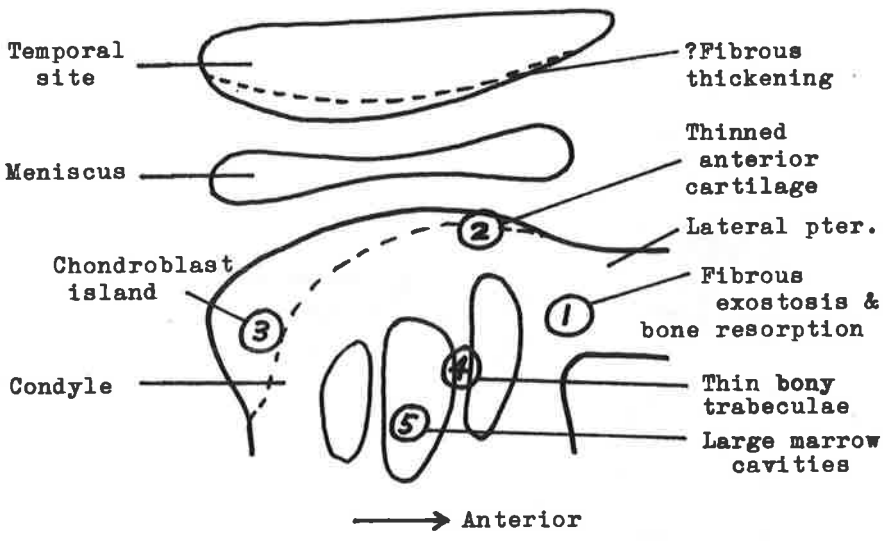
In some areas, deposition of osteoid and bundle bone has occurred at areas of bone resorption (Fig. 11.6 on p.11.4).

(4) Posteriorly, the transitional layer of the fibrous covering is still present, and the cartilage layer is thicker than in the anterior region (about 5 cells thick). The whole layer of cartilage in this posterior region appears normal except in one area: in this region, the continuity of the cartilage has been disrupted by an island of small chondroblasts which have replaced an area of the underlying bone. This island is in direct contact with condylar bone in its deep aspect, but anteriorly it merges into the rest of the normal cartilagenous covering (Fig. 11.4 on p.11.3).

(5) The marrow cavities in the lathyritic condyle are much larger than normal, although their shape and content (the marrow cells) are unchanged (compare Fig. 11.1 on p.11.2 with Fig. 1.11 on p.1.18 for different sizes of marrow cavities).

All of these changes in the lathyritic joint were apparent from between 4 to 8 weeks after feeding on the diet. The only exception is the formation of the fibrous exostoses, the first signs of which were observed in 1-week rats. It took place at the anterior aspect of the condyle (at the attachment of the lateral pterygoid muscle) where, at places, the normal periosteum is replaced by a slight thickening of cellular tissue consisting of many fibroblasts in line with the pull of the muscle.

A summary of lathyritic changes in the joint is shown in the following line-drawing:



# CHAPTER 12

## DISCUSSION : SERIES " L "

In the literature on the effect of lathyrism on the temporomandibular joint structures, only Gardner (1960) and Gardner et al. (1958) mentioned any change in the temporal site. They recorded deposition at the "lip" of the temporal bone thereby deepening the gutter.. The only change observed in the present investigation is a slight thickening at the lateral lip due to the replacement of bone by fibrous tissue, i.e. a thickening of the fibrous covering at the expense of bone. No bony deposition as reported by Gardner has been observed, nor was it recorded by Krikos et al. (1958) and Sarnat et al. (1965). In the present study, fibrous thickening was observed in one rat only, and as mentioned before (p.11.1), it could be due to oblique sectioning of the particular specimen, and therefore may be regarded as of doubtful significance.

With regard to condylar "exostoses" the writer would support the observations of the previous workers. Exostoses were certainly seen at muscle attachments, and the histology was similar to that reported previously (Gardner 1960, Gardner et al. 1958, Krikos et al. 1958, Sarnat et al. 1965), viz. the very cellular arrangement of spindle cells and immature collagen, the attachment to bony surfaces being associated with active resorption of bone.

The general appearance of the lathyritic condylar cartilage is as follows: (1) anteriorly it is thinner than

normal, but regular, and (2) posteriorly it is irregular, with an irregular arrangement of chondrocytes and hyaline material surrounding them. Clearly the report by Krikos et al. (1958) was correct and their coronal sections must have been obtained in the posterior part of the condyle. With this general picture in mind, the controversial two results of Gardner et al. (1958) can be explained: In his "first group" of rats, with "continued chondrocyte proliferation and calcification" but of irregular appearance and arrangement, the condyle must have been sectioned at the posterior part in the coronal plane. In his "second group" of rats, with "thinning of the condylar cartilage and eventual disappearance", the condyle must have been sectioned coronally at the anterior part. This again illustrates the important observation made in Chaper 7 (p.7.5), concerning the need for a comprehensive picture of the joint by sagittal sections as well as coronal sections, as coronal sectioning at the anterior part of the joint gives a different picture from that found at the posterior part of the joint. The sagittal sections made in the present investigation explain the difference in results obtained in Gardner's two series on the lathyritic condylar cartilage.

As for condylar bone, the present findings agree with those of previous workers, viz. thinning of trabeculae associated with increased resorption, and an increase in the size of the marrow spaces.

No change in the temporal bone or in the articular meniscus was observed in the present study. The previous workers also recorded no changes in these structures.

With these observations in mind, it is now possible to answer the questions asked in Chapter 9 (p.9.6):

(1) The long term changes in the joint are: 1. the formation of cellular exostoses on the condyle, 2. thinning of the condylar cartilage anteriorly, 3. thickening and irregularity of the cartilage posteriorly, 4. thinning of bony trabeculae with resorption, and 5. associated widening of marrow spaces.

(2) The final changes in the condylar cartilage are as described above. The two different findings of Gardner et al may be explained by their sectioning of the condyle in the coronal plane only, in one case in the anterior region, and in the other in the posterior region.

(3) & (4) No changes were observed in either the articular meniscus or the temporal site after 12 weeks of feeding.

(5) Both sagittal and coronal sections were made in the present study, which gave a more accurate picture of changes in the joint than was possible with the methods used by previous workers on this subject.

In view of the changes in the periodontal fibrous tissues observed (p.9.4) and the lack of these changes in the fibrous tissues of the articular meniscus, some explanation is necessary. Although lathyrism weakens collagen structurally, actual physical stress must be present to disrupt it. In the periodontal membrane of functioning teeth constant masticatory stress is present which readily induces observable changes; however when this stress is absent, as in the periodontal

membrane of an unerupted lower third molar, changes are minimal or absent (Krikos et al. 1958). The absence of changes in the articular meniscus of the temporomandibular joint illustrates an important feature, which is that in the normally functioning joint there is little or no physical stress on the meniscus.

As it has been shown that in the absence of stress no changes have taken place in the joint on a lathyrogenic diet (except for disturbance of growth in the condylar cartilage), the possible effects of stress on such a joint created by mandibular overclosure has been investigated, and this is the subject of the next Section of this thesis (Section IV).

## SECTION IV

# THE EFFECTS OF LOSS OF POSTERIOR OCCLUSION ON THE LATHYRITIC JOINT

# CHAPTER 13

## PLANNING OF EXPERIMENTAL SERIES " LE "

In this series of experiments, there is only one variable compared to Series "L", i.e. the posterior teeth in the upper jaw were extracted to produce overclosure.

10 Sprague Dawley rats were subjected to a maxillary posterior dental clearance (under general anaesthesia) in the same way as in Series "E" (Chapter 5 and Appendices II & III). However, on recovery, they were fed on a lathyrogenic diet instead of a normal diet.

The various time intervals after which rats were sacrificed for examination were as follows:

| Time (from extractions and start of diet) | No. of rats | Laboratory designation |
|---|---|---|
| 2 days | 2 | LE |
| 1 week | 2 | LE1 |
| 4 weeks | 2 | LE4 |
| 8 weeks | 2 | LE8 |
| 12 weeks | 2 | LE12 . |

The method of using controls is discussed in Appendix I.

This series was designed to follow the same pattern as Series "L", so that any difference noted in the results

between these two series could be related to the loss of posterior occlusion. Again, the rationale for not running this series for longer than 12 weeks and for using a "50/50" mixed lathyrogenic diet is applied for the same reasons as in Series "L" (c.f. pages 10.1 - 10.2).

At least one joint from each rat was examined, some sectioned sagittally, some coronally. Photomicrographs were taken from each series of sections, mounted on a large piece of cardboard adjacent to those of the other series, and compared with them in order to observe pathological changes at the respective time intervals.

# CHAPTER 14

## RESULTS OF EXPERIMENTAL SERIES " LE "

The same parameters were used in the examination of sections as those used in Series "E" (Chapter 6).

### TEMPORAL SITE

Changes at the temporal site were observed after 12 weeks of feeding with the lathyrogenic diet:

(1) The fibrous covering of the temporal bone was normal until 12 weeks when its structure became irregular in the lateral part of the joint (Fig. 14.3). In one area in the lateral part there was bone resorption with replacement by fibrocytes which were arranged in the form of a circular mass.

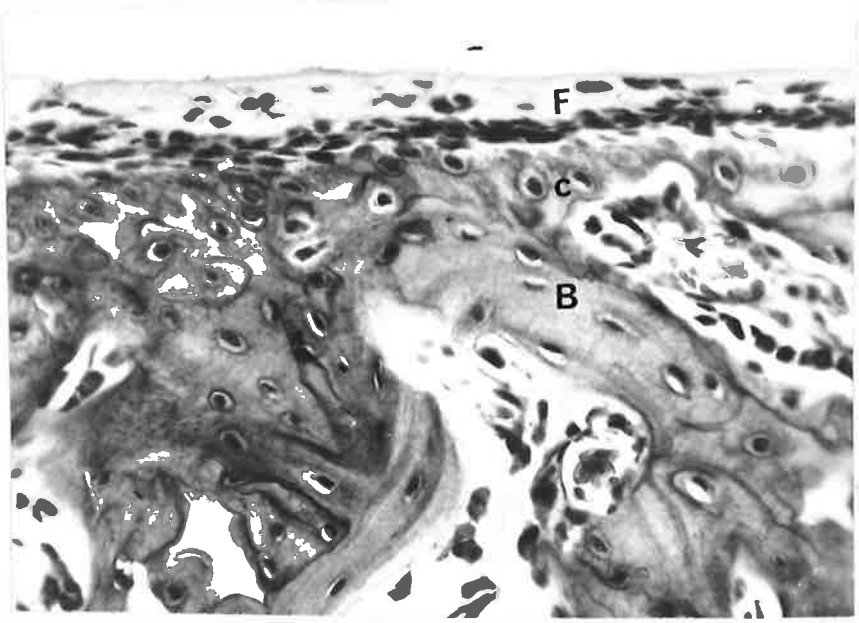
(2) The bony trabeculae surrounding the marrow cavities were much thinner than normal (Fig. 14.1).

(3) The marrow spaces became very large after 12 weeks, and the haemopoietic content in them was increased (Fig. 14.1), with the cells totally filling the spaces.

Fig. 14.1 SERIES "LE", sagittal section.
H & E x25

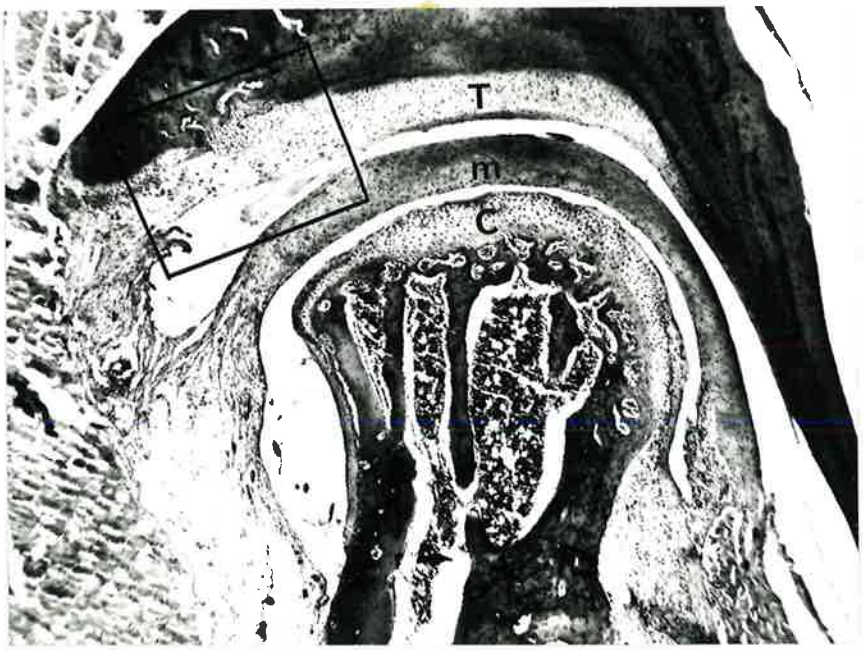

T = Temporal site
M = Meniscus
C = Condyle
E = Exostosis

Fig. 14.2 SERIES "LE", Thinned anterior condylar cartilage.
H & E x250




C = Cartilage
F = Fibrous covering
B = Bone

Fig. 14.3 SERIES "LE", coronal section.
H & E x25




T = Temporal site

M = Meniscus

C = Condyle

Fig. 14.4 SERIES "LE", Temporal Bone resorption in lateral region.
H & E x100


R = Resorption area

MENISCUS

No change was seen in the meniscus up to 12 weeks.

CONDYLE

Many changes in the condyle were observed:

(1) The surface of the condyle became irregular. This took the form of an undulating overall convexity starting from the middle extending posteriorly and being most pronounced in the most posterior part of the condyle (compare Fig. 14.3 with Fig. 1.12 & Fig. 6.3; and Fig. 14.1 with Fig. 1.18).

(2) Severe bone resorption was present at the attachment of the lateral pterygoid muscle so that particularly at the antero-lateral portion of the condyle there was only a small area of bone with a large fibrous exostosis associated with it (Fig. 14.1).

(3) The histology of the exostoses is similar to that found with lathyrism alone, and this has been described on page 11.1.

(4) The fibrous covering of the condyle was overall thinner and irregular in thickness. Anteriorly it was seen to be disrupted in one area where the layer of fibrocytes was replaced by an oblique sheet of dense collagen (Fig. 14.5).

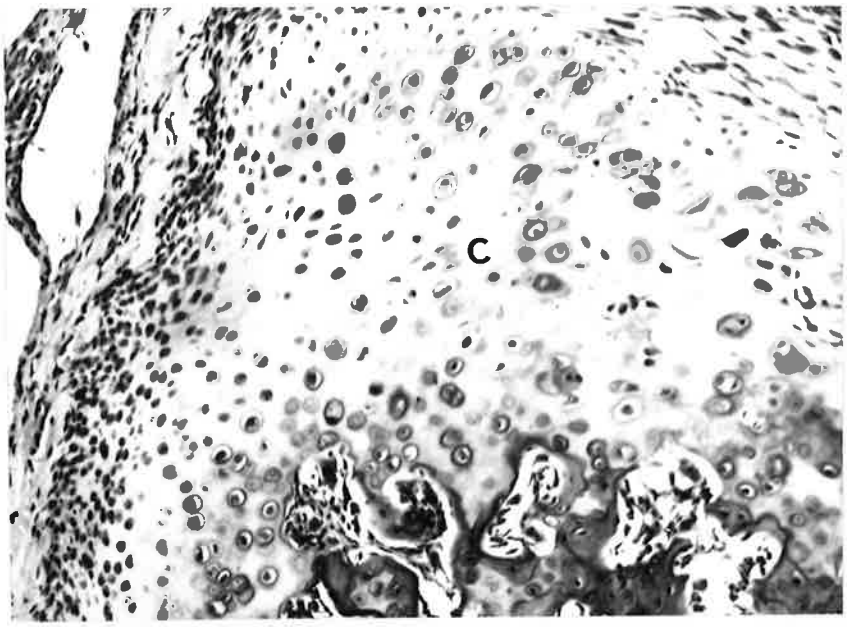
(5) In the coronal plane the fibrocytes of the surface layer were seen to clump into groups, in some cases as groups of 5 or 6 cells, and in some up to 11 or 12 cells.

Fig. 14.5 Layer of fibrocytes at anterior part of condyle disrupted by oblique sheet of dense collagen

H & E x100

M = Meniscus
A = Area of discontinuity of fibrocytes
C = Condyle

Fig. 14.6 Irregular posterior condylar cartilage at 12 weeks.
Sagittal section, H & E x100
C = Cartilage




(6) The condylar cartilage became very irregular in thickness after 12 weeks, the first change being apparent after 4 weeks. Anteriorly the layer was as thin as 1-2 chondrocytes thick, but posteriorly the cells were arranged in whorls with hyaline areas here and there, and the thickness of cartilage varied from 14 cells thick to 2 cells thick in adjacent areas (Fig. 14.6).

(7) The bony trabeculae were irregular and thin.

(8) The marrow spaces were very large, and the content consisted of an increased amount of haemopoietic tissue.

In summary, most of the changes in these post-extraction rats were readily observed after 4 weeks of feeding on the lathyrogenic diet, and progressed steadily to 12 weeks. The exostosis at the attachment of the lateral pterygoid muscle appeared much earlier, being observed after one week.

# CHAPTER 15

## DISCUSSION : SERIES " LE "

In contrast with the results observed with rats fed on lathyrogenic diet alone, in the post-extraction rats on the same diet there were changes in the temporal site. Resorption in the lateral wall of the temporal site is a significant finding, because it is probably related to a change in masticatory pattern, as is suggested in the discussion later.

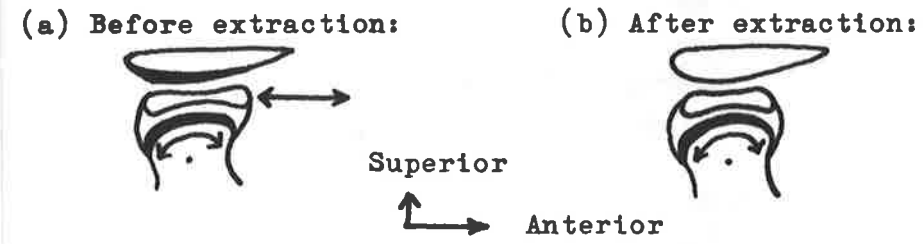
As for the resorption within the substance of the bone and the resultant extremely large marrow cavities, it is difficult to relate stress to these changes as previous workers (Krikos et al. 1958, Gardner et al. 1958) have reported similar changes with lathyrism alone.

With the articular meniscus, there was no change in shape or in histological structure. This result tends to support the observation that no functional stress is placed on the joint with mandibular overclosure and associated change in masticatory pattern. How then can one reconcile the bone resorption seen at the temporal surface? An analysis of the change in masticatory methods of the rat after posterior dental clearance may give us an understanding of the underlying causes of this change:

Before extractions, the rat used antero-posterior movements (molars chewing) as well as opening and closing

(incisors); after posterior extractions, this was changed apparently to opening and closing only (incisors), as observed by the writer on the eating habits of rats in the animal house:

Fig. 15.1 MOVEMENTS OF MENISCO-CONDYLAR COMPLEX:




It is possible that after extraction, the lack of horizontal "rubbing" by the menisco-condylar complex on the temporal bone removed the normal functional stimulus on it. The result of this is bone resorption (Disuse atrophy) at the temporal site. The meniscus, however, still had a functional "rubbing" by the condyle below. It is therefore understandable that while bone resorption was observed on the temporal site, no structural change was seen on the meniscus after this experimental period. The bone at the temporal site is probably more labile than the meniscus.

As for the mandibular condyle, the surface became quite irregular after 12 weeks, in contrast to the smooth surface after a similar period of lathyrism alone. Once again this may mean that lack of posterior occlusion and mastication tended to produce changes in the condylar surface due to changes in the masticatory pattern.

Other changes in the condyle such as the thickening of posterior cartilage and its irregularity, thinning of cancellous spicules and widening of marrow spaces are similar ro those produced by lathyrism alone except that they developed earlier. In this series ("LE") most changes were obvious by 4 weeks, whereas with lathyrism alone results were not obvious until after 8 weeks. This could be due to disuse atrophy at the temporal site and condyle resulting from lack of normal functional stimuli in the Joint.

Therefore, by way of comparison with lathyrism alone, lack of posterior occlusion on lathyritic joint structures produced the following:

(1) There was evidence of temporal bone resorption at the surface and internally, at the end of 12 weeks. This was not seen with lathyrism alone, and could therefore be suggested to be due to the occlusal disharmony as a result of posterior extractions.

(2) At the end of 12 weeks, the condyle surface was irregular.

(3) In the posterior region of the condyle there was cartilage irregularity, resorption of condylar bone, and widening of marrow spaces, all of which were seen much earlier than with lathyrism alone (4 weeks compared with 8 weeks).

# SECTION V

# SUMMARY AND CONCLUSIONS

## SUMMARY AND CONCLUSIONS

The present study was designed to investigate:

(1) Functional occlusion in the Rat and the part played by the temporomandibular joint;

(2) The structure of the lateral pterygoid muscle and its functional role with respect to harmony between the mandibular condyle and the articular meniscus, with particular reference to its controversial "two components";

(3) The effects of lathyrism on joint structure; and

(4) The effects of loss of posterior occlusion on joint components under the following conditions, from 2 days to 12 weeks:

    (a) normal function, and

    (b) following weakening of collagen by lathyrism.

To provide an answer to (1) and (2), gross dissection and histological studies were undertaken. The presence of two components of the lateral pterygoid was confirmed and its relationship with joint structures was compared with that in other animals. To provide an answer to (3) and (4), four series of histological studies have been undertaken, one control series, one with maxillary molars removed, one with lathyrism only, and one with maxillary molars removed and fed on a lathyrogenic diet. Each series consisted of 10 rats, making a total of 40 rats, and histological examination consisted of serial

sectioning of the temporomandibular joints in both the sagittal and the coronal planes in each series of rats.

It was found that:

1. The anatomy of the temporomandibular joint was closely related to the specialised functional requirements for mastication.

2. The lateral pterygoid muscle of the rat was found to be intermediate in structure and function between the rabbit and the Primates, and on the basis of comparative anatomy, the evidence strongly supports the "two muscle" concept for the human lateral pterygoid as put forward by Christensen (1969). In comparison with the human joint where movement takes place at different speeds in a co-ordinated fashion, the meniscus in the rat moves at a similar speed to that of the condyle due to the simpler form of the joint (p.4.5) as related to masticatory movements; nevertheless it is still a co-ordinated movement.

3. With loss of posterior occlusion in rats on a normal diet, no change in any joint component was noted up to 12 weeks. Alleged histological changes reported by previous workers have been disproved.

4. With lathyrism, changes were noted in the mandibular condyle as follows:

   (a) Bone resorption and fibrous "exostosis" formation at the attachment of the lateral pterygoid;

(b) Thinning of anterior condylar cartilage;

(c) Formation of a chondroblast island in the posterior region of the condylar cartilage;

(d) Thinning of bony trabeculae; and

(e) Enlargement of marrow cavities.

5. In the lathyritic joint following loss of posterior occlusion, the following results were noted:

(a) Temporal bone resorption at 12 weeks;

(b) Irregularity of posterior condylar surface at 12 weeks;

(c) In addition, those changes noted in paragraph "4." above were noted.

6. There was no change in the articular meniscus in any of the experimental series.

The above results confirm that changes in collagen structure occur under stress, and suggest that the temporomandibular joint is not a stress-bearing joint as only under conditions of stress have changes taken place.

The present study emphasises the importance of sagittal sectioning of the temporomandibular joint in investigating structural changes. Due to the long anteroposterior dimension of the joint, all the joint structures present variations at different levels in the coronal plane. The use of coronal sections only by previous workers has been shown to have led to erroneous interpretations. No doubt the difficulty of obtaining sagittal sections due to

the narrowness of the joint medio-laterally and the consequent difficulty of accurate orientation, has led to the use of coronal sections only by previous investigators.

# APPENDIX I

## MATERIALS, METHODS, AND PLANNING OF EXAMINATION OF NORMAL RATS

In order to establish the normal anatomy and histology of the Rat Temporomandibular Joint and associated structures, a series of 10 Sprague Dawley rats were chosen. These were sacrificed and examined at various time intervals to provide controls for the experimental series. The times when they were sacrificed paralleled those of the experimental ones, and were as follows:

| Time (from start of experiments) | Rats | Laboratory designation |
|---|---|---|
| 2 days | 2 | C |
| 1 week | 2 | C1 |
| 4 weeks | 2 | C4 |
| 8 weeks | 2 | C8 |
| 12 weeks | 2 | C12 |

The rats were sacrificed by using an overdose of Nembutal. The heads were decalcified and the temporomandibular joints removed for examination (Appendices II & V).

As the temporomandibular articulating surfaces and the meniscus were the chief objectives of this study, one joint from every normal rat was critically examined at the respective times. Some joints were sectioned

sagitally, some coronally. This was to provide a good overall picture in two planes of section.

The muscles of mastication were studied by dissection of a Rat's head which had been fixed in formalin for 3 days for better definition of muscles and fasciae. The muscles were dissected proceeding from superficial to deep, i.e. masseter, temporalis, medial pterygoid, lateral pterygoid, and anterior belly of digastric, in that order. Attention was paid to their size, shape, origin and insertion, number of origins and insertions, and the direction of muscle fibres.

All dissections of muscles were repeated using a second normal rat. Any further observations were made, and all previous observations were checked, corrected, and confirmed.

The teeth and their occlusion were studied together with the examination of the muscles of mastication.

Finally, dry skulls were examined to check and confirm the morphology of the temporal articular surface, the mandibular condyle, the teeth and occlusion, the origin and insertion of the masticatory muscles, and their relationships with each other.

# APPENDIX II

## SURGICAL ANAESTHESIA AND POST-OPERATIVE CARE

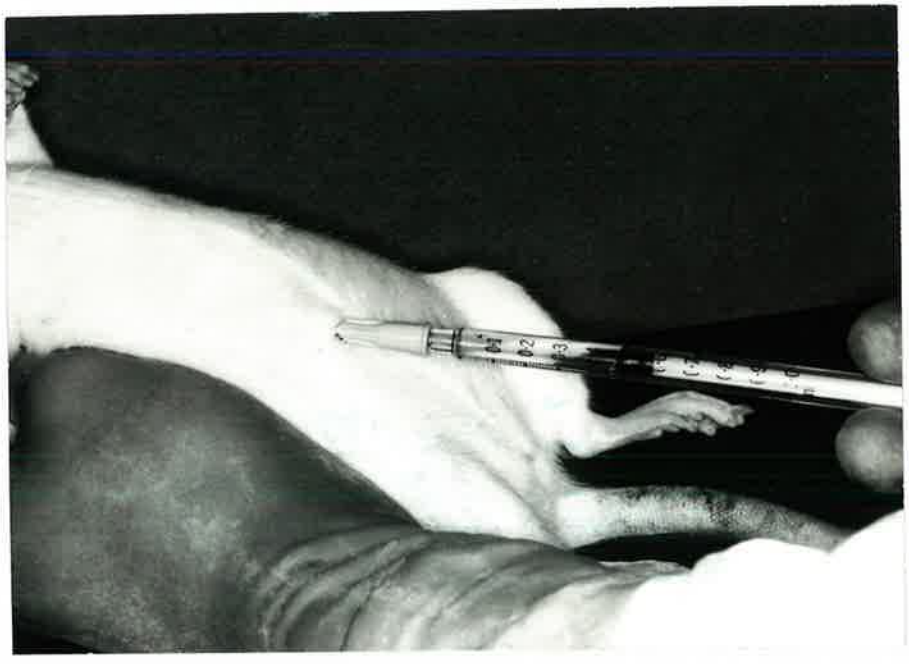
For surgical anaesthesia, Pentobarbitone sodium (Nembutal) was used following the manufacturer's recommended dosage of 1 ml. of the 6°/. solution (veterinary, 60 mg./ml.) per 5 lb. body weight.

The experimental rats were all approximately 8 ozs. in weight, the variation being extremely small. Each rat would therefore require 0.1 ml. of the 6°/. solution of Nembutal. Experience in the Department of Oral Pathology and Surgery in the past suggested, however, that the use of undiluted 6°/. solutions in small animals produced a moderate mortality rate (Smale 1972). Therefore in this study the 6°/. solution was diluted with normal saline, Nembutal : Normal saline = 1:9 (i.e. diluted 10 times). The dosage used for surgical anaesthesia with this diluted solution would then be 1.0 ml.

ANAESTHETIC ARMAMENTARIUM:

The rats were placed first in an ether chamber until they became drowsy and easily manageable. The pentobarbitone was then administered by intraperitoneal injection. The rats first recovered slightly from the ether, but within 10 minutes all reached a sufficient depth of anaesthesia from the pentobarbitone, and were then ready for the extraction of teeth.

INTRAPERITONEAL INJECTION:



Care was taken during the operation to avoid aspiration of blood. This was done by meticulous gentle suction or by the use of cotton gauze swabs. Aspiration of blood into the lungs was a real danger as some rats tended to have bouts of sudden and profound gasps while under the anaesthetic.

Post-operative care included suction which in some cases was necessary, the protrusion of the tongue to ensure a clear airway, and the placement of the head in a suitable position for drainage of blood by gravity.

Recovery from the anaesthetic was gradual and took an average of 2 hours to complete.

SACRIFICING FOR EXAMINATION

Sacrificing of rats was effected by intra-peritoneal injections of 1.0 ml. of the undiluted 6% Nembutal solution, i.e. 10 times the anaesthetic dose. Respiration would stop after an average of 5-10 minutes. While not wishing to remove the heads before the rats were completely dead, this procedure was not left for too long because autolysis would start immediately after death. A compromise time interval from cessation of respiration to the removal of the heads was 2 minutes.

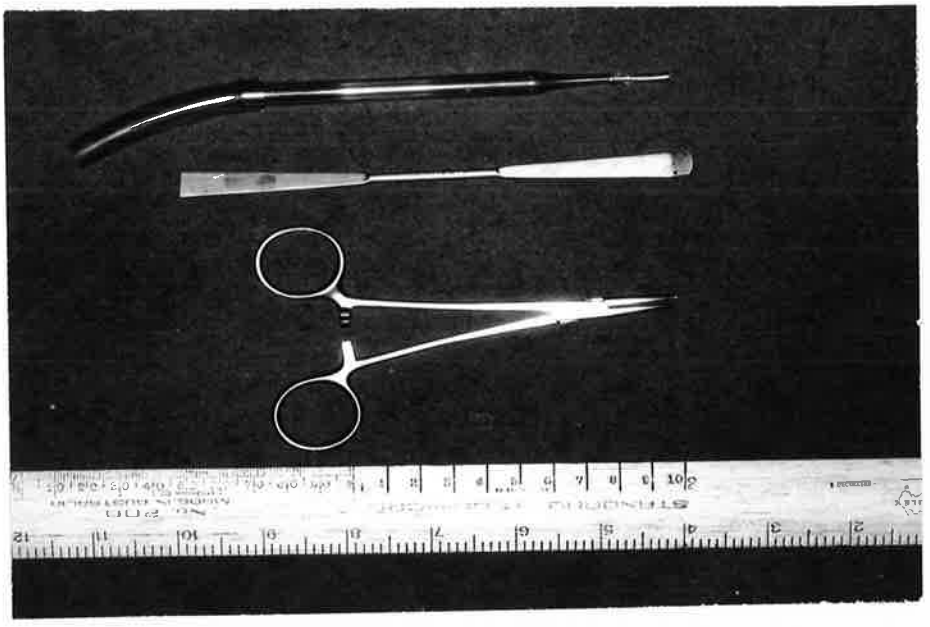
# APPENDIX III

## METHOD OF EXTRACTION OF POSTERIOR TEETH IN RATS

### ARMAMENTARIUM

A fine-tipped sucker
A spatula (used as cheek retractor)
A modified mosquito artery forceps
The operating table.

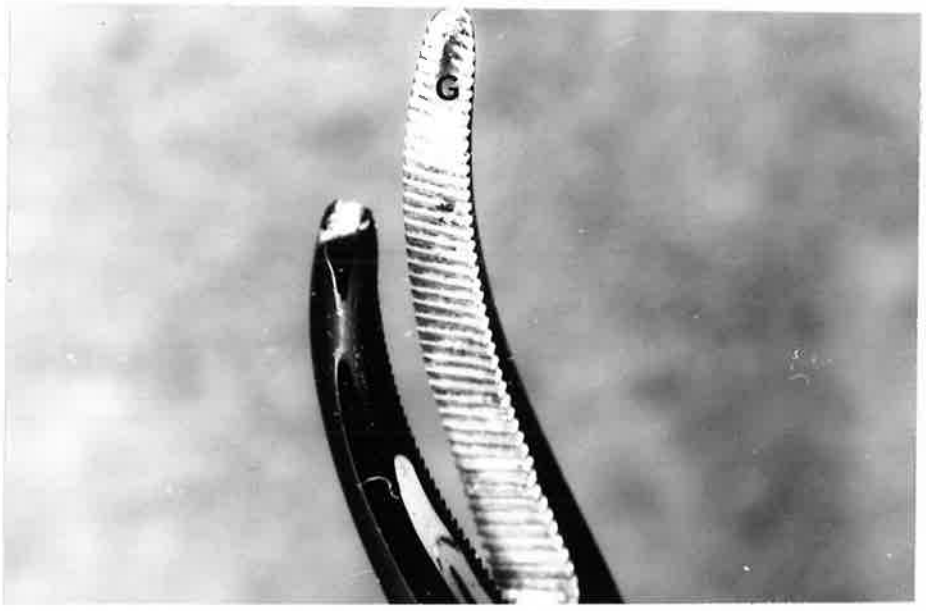
SURGICAL ARMAMENTARIUM:



The modification of the Mosquito Artery Forceps was devised by the writer. The fine beaks of the ordinary mosquito forceps suited the size of the rat molar teeth well, but did not provide a firm grip. A longitudinal groove was therefore made in the middle of each beak so that a concavity was formed on each, and both beaks together would mould around the crowns of molars and thereby offer a firm hold.

THE RAT MOLAR FORCEPS:

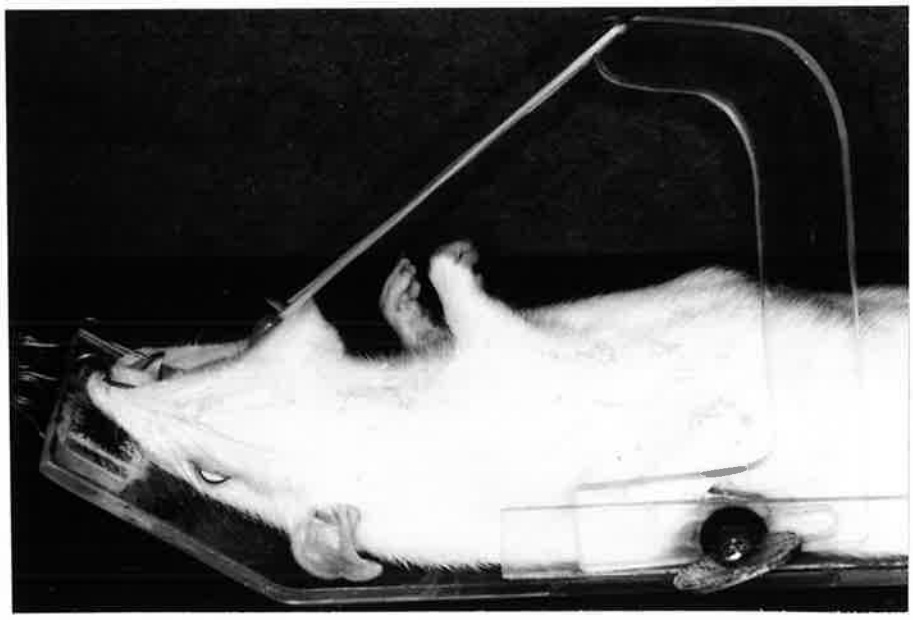
G = Groove



## POSITIONING THE RAT

The operating table had a head portion which was fitted with a spring-wire and a vertical arm which was notched for insertion of an elastic band. The spring-wire hooked onto the upper incisors of the anaesthetised rat and helped to open its mouth. The elastic band hooked from the vertical arm of the table to the lower incisors of the rat thereby holding its mouth open. The rat was then ready for the operation.

RAT IN OPERATING POSITION:



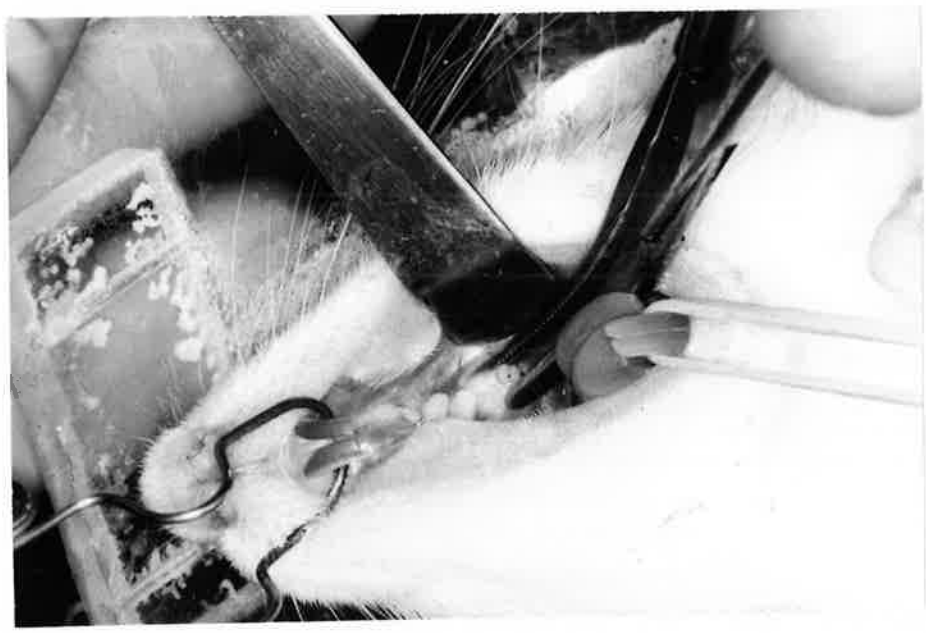
## EXTRACTION OF MOLARS

With the assistant retracting the cheek with the spatula and sucking the blood when necessary, the operator removed the teeth with the modified forceps. The first molars were the most difficult to extract and often

fractured, and in this case the separate portions of the teeth would require individual removal, but the roots were generally not firmly anchored and could therefore be easily removed. The second and third molars, on the other hand, were relatively easy to remove.

The average duration of maxillary posterior clearances was less than two minutes. Quick and accurate manoeuvres were essential to provide cleanliness of wounds and a minimal amount of bleeding. Immediate post-operative care has been discussed in Appendix II (on p.A.5).

EXTRACTING MOLAR TEETH:



# APPENDIX IV

## THE LATHYROGENIC DIET

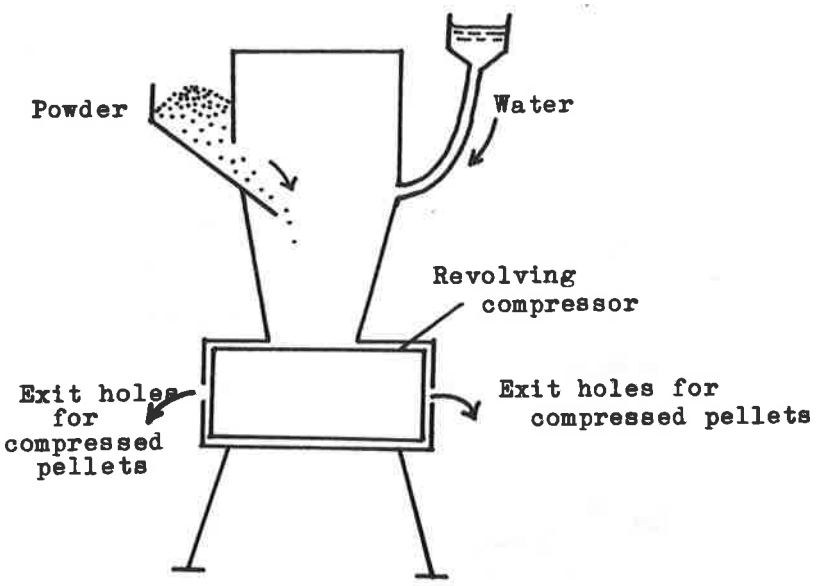
The active ingredient used in the lathyrogenic diet was Sweet Pea seeds, Lathyrus odoratus (Bachhuber & Lalich 1955, Levene 1961, Levene & Gross 1959). These seeds were purchased from a local green grocer's, but the seeds were originally imported from New South Wales.

As an entire sweet-pea meal might deprive the rats of ordinary essential nutriments, a 50/50 diet was used where the seeds were mixed with ordinary rat food. To effect homogenious mixing, both ingredients needed grinding into powder and then thoroughly blended. This was done by courtesy of the Department of Botany, University of Adelaide. The ingredients of ordinary rat food pellets were:

| | | |
|---|---|---|
| Yeast | Butter milk powder | Soya |
| Lucerne | Meat | Wheat |
| Bone flour | Mollases | Fish |
| Barley | Salt | Vitamins |
| Bran and pollard | | |

The mixture in fine powder form would not, however, stimulate mastication adequately, and therefore had to be made into hard pellets. This was done with a pelleting machine made available by the Northfield Agricultural Research Institute, South Australia. The structure of the machine is shown in the drawing below:

THE PELLETING MACHINE:



# APPENDIX V

## TEMPOROMANDIBULAR JOINT DISSECTION EN BLOC

Removal of the temporomandibular joints en bloc for histological processing required the following:

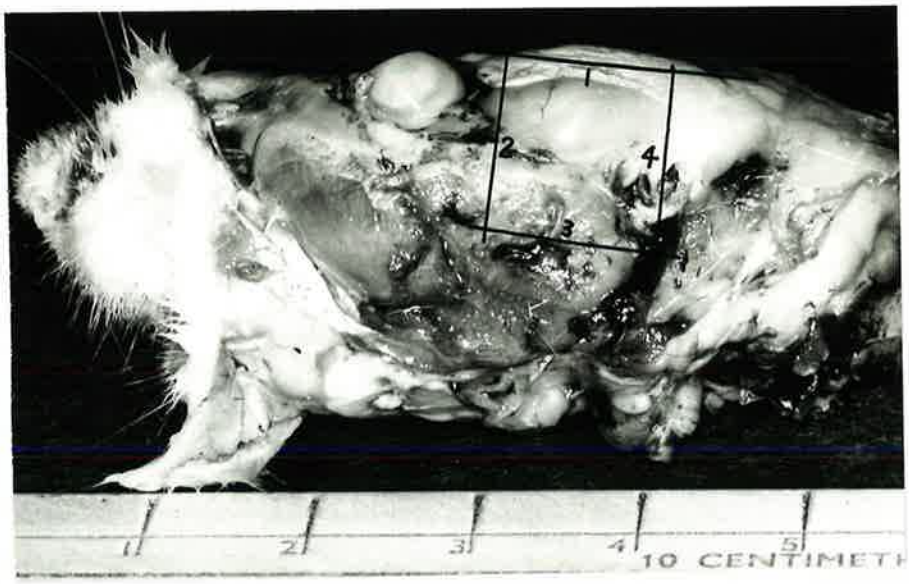
(1) Accuracy: if the block removed was large there was a better chance of not missing the joint, but it would mean a need for longer decalcification and would produce clumsiness during sectioning and mounting; if the block was too small, one might cut into the joint itself during removal thereby damaging it and creating artefacts in the block. The ideal was to remove as small a block as possible while including the whole joint.

(2) Minimum trauma: to preserve the original status quo of the joint structures, a minimum of instrumentation during the dissection was essential. The use of a dental engine and bur would create too much vibration while cutting the bony structures; hence the heads of the rats required initial softening by decalcification (Appendix VII) which would then necessitate no more than a scalpel blade for the whole procedure of removal of the joint en bloc.

A knowledge of the anatomy of the rat's skull and experience with removal of temporomandibular joints from the first few rats using different methods, taught the writer to adopt the following steps for a clean and accurate dissection:

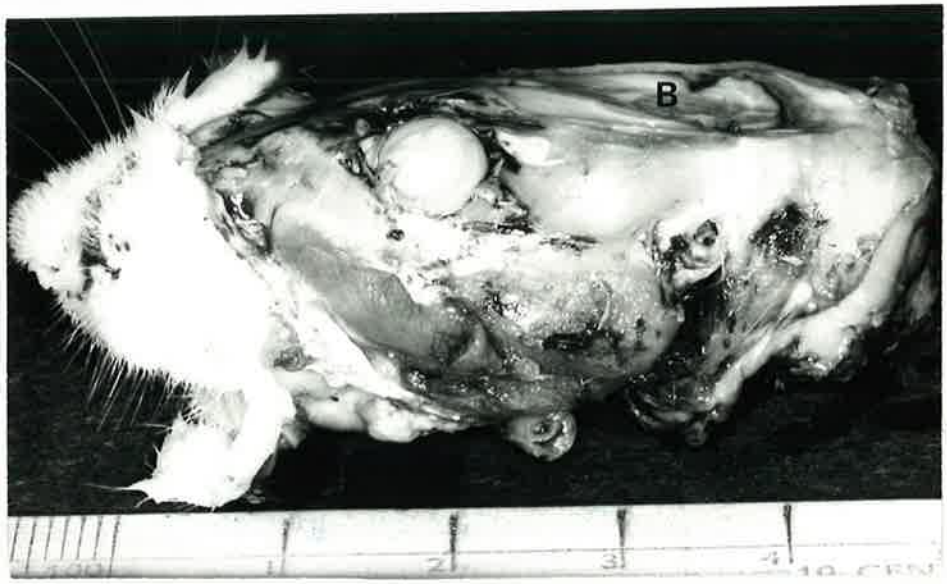
(1) INCISION LINES: The landmarks on the lateral aspect of the head are: the eye, the lower border of the mandible, the external auditory canal, and the superior surface of the head (Fig. V.1):

Fig. V.1 INCISION LINES FOR REMOVAL OF JOINT:




(2) The first cut was to plane the superior 2 mm. from the surface as shown in Fig. V.1. The result is shown in Fig. V.2, where the brain is now visible.

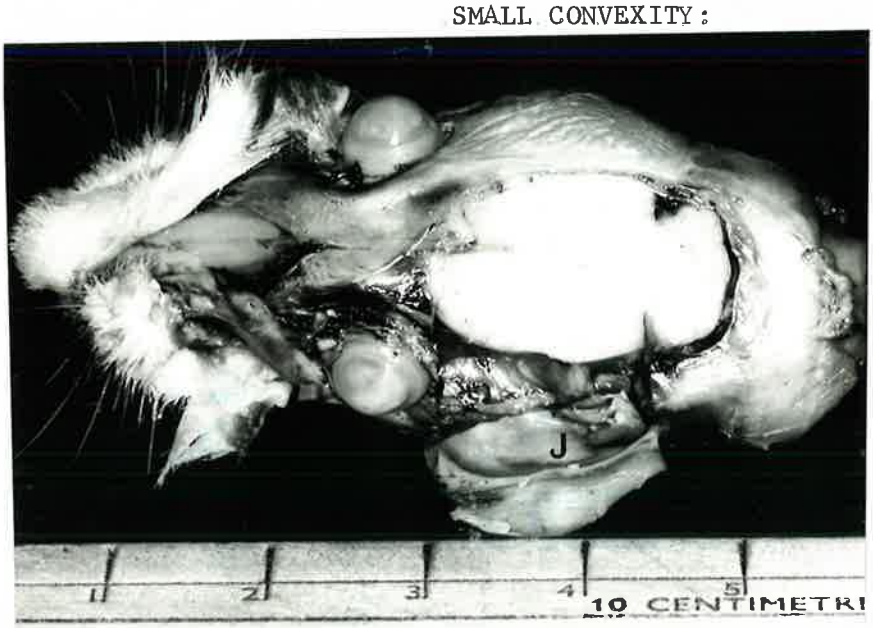
Fig. V.2 AFTER THE FIRST CUT: B = Brain



(3) The next step was to cut along Lines 2, 3, and 4 as shown in Fig. V.1 at a depth of 7 mm from the surface.

(4) The plate of temporal bone (and some of parietal bone) including the most medial aspect of the temporomandibular joint could now be detached from the brain, revealing the site of the joint as a small convexity near the centre of the plate (Fig. V.3):

Fig. V.3 MEDIAL ASPECT OF JOINT VISIBLE AS A SMALL CONVEXITY:




J = Joint

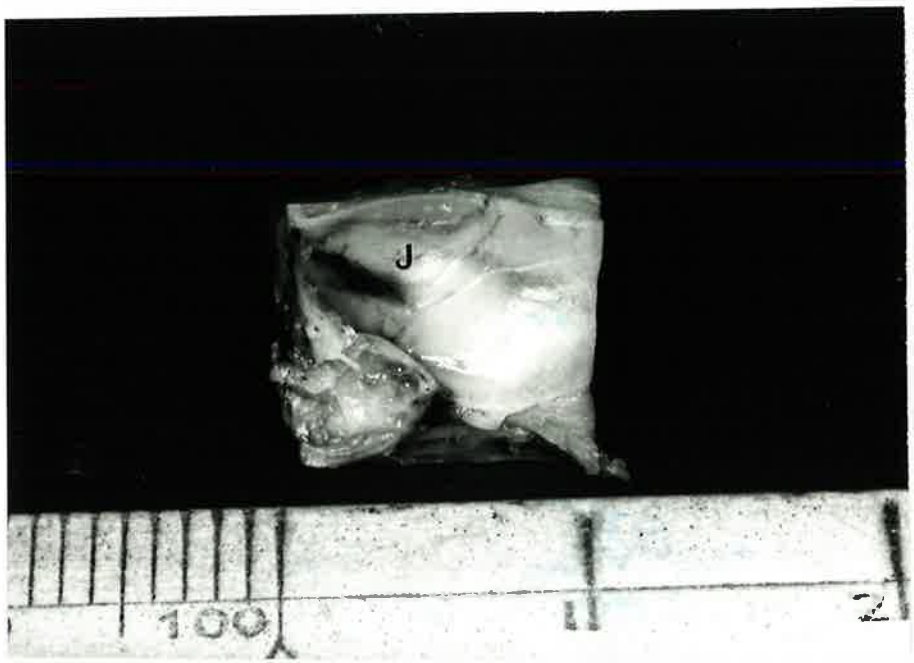
(5) The block, completely removed, could now be trimmed further if desired using the convexity as the guide. Its correct orientation and site (convexity) of the joint are shown in Fig. V.4 (next page).

Once trimmed, the joints were replaced in decalcifying fluid for completion of the process of decalcification (Appendix VII).

Fig. V.4 THE TEMPOROMANDIBULAR JOINT IN THE BLOCK REMOVED FROM THE HEAD:

(Viewed from medially)

J = Joint area (convexity)



# APPENDIX VI

## TISSUE FIXATION : FORMOL SALINE

| | | |
|---|---|---|
| FORMOL SALINE | Formaldehyde solution 37-40°/₀ | 100 ml. |
| | Sodium chloride | 8.5 gm. |
| | Tap or distilled water | 900 ml. |

Fixation in formaldehyde could be influenced by the concentration of the reagent and by temperature. Adequacy of fixation has to be ensured, particularly if the specimen is large. Higher temperatures will bring about faster fixation, but autolysis will also be hastened. The use of temperatures above 60°C involves the factors of heat coagulation and of loss of formaldehyde through volatilization. Higher concentrations of formaldehyde will fix faster, but will tend to over-harden tissues and to affect staining adversely.

In this study, the heads of the experimental rats were fixed for 3 days in the solution shown above. Fixation was at room temperatures.

# APPENDIX VII

## HARD TISSUE DECALCIFICATION

### DECALCIFYING SOLUTION (SCHMORL'S FORMULA):

| | |
|---|---|
| Formic acid 90%/o | 500 ml. |
| Formaldehyde 40%/o | 50 ml. |
| Distilled water | 450 ml. |

Tissues undergoing decalcification were provided with fresh decalcifying solution each day. In the present study, the heads of the experimental rats were preliminarily decalcified for 3 days to allow easy removal of the temporomandibular joints using a scalpel (Appendix V), without risking the danger of disturbing or damaging them as would result from the use of burs. The joints were then replaced in decalcifying solutions for completion of the process of decalcification. As they were now much smaller in size compared to the whole rat's head, this process took a shorter time to complete (a further 3 days).

Checking the absence of residual calcium in the bony tissues can be done by reaction with sodium or ammonium oxalate, precipitating calcium oxalate. However, the method adopted in this study was to use radiographs: an absence of radio-opacity suggested the absence of calcium in the bones, i.e. decalcification completed.

# APPENDIX VIII

## EMBEDDING PROCEDURES

After decalcification the specimens were neutralised in sodium sulphate 5°/₀ for about 12 hours before embedding. The specimens then went through the following reagents:

| | | | |
|---|---|---|---|
| (A) | 37°C: | 1. Alcohol 70°/₀ | 1 hour |
| | | 2. " 80°/₀ | " |
| | | 3. " 90°/₀ | " |
| | | 4. " 95°/₀ | " |
| | | 5. " absolute | " |
| | | 6. " " | " |
| | | 7. " " | " |
| | | 8. Absolute alcohol & methyl salicylate (1:1) | " |
| | | 9. Methyl salicylate & celloidin 0.5°/₀ | 2 days |
| | | 10. " 1°/₀ | " |
| (B) | 60°C: | 11. Wax & Methyl salicylate (1:2) | 1 hour |
| | | 12. " " " (1:1) | " |
| | | 13. " " " (2:1) | " |
| (C) | 56°C: | 14. Wax | 1 hour |
| | | 15. " | " |
| | | 16. " | overnight. |

The specimens were then placed in a vacuum for 1 hour for evacuation of air bubbles, and were subsequently blocked in wax (at 56°C).

It was noted in some of the early blocks that although the specimens were put in a vacuum, wax failed to infiltrate completely into the joint cavities because they were partially sealed by surrounding structures (such as the temporal bone medially and masseter laterally). The result was brittleness of the sections or, at times during sagittal sectioning, detachment of part or whole of the mandibular condyle from the remainder of the block. This problem was overcome by first cutting into the joint cavities with the microtome thereby allowing communication between these cavities and the surface of the block, and then re-vacuuming for another hour before finally reblocking.

Blocks prepared by the re-vacuuming method gave no further problems.

# APPENDIX IX

## STAINING TECHNIQUES

### DEWAXING AND HYDRATION

The histological sections went through:

| | |
|---|---|
| Xylol | 2 minutes |
| Absolute alcohol | " |
| Alcohol 70°/₀ | " |
| Distilled water | 1 minute. |

### DEHYDRATION AND MOUNTING (after staining)

| | |
|---|---|
| Absolute alcohol | 1 minute |
| " " | " |
| Alcohol-Xylol | ½ minute |
| Xylol | " |

Mounted in DPX with cover-slide.

### STAINS EMPLOYED

The staining techniques used in this study were:

(1) Haematoxylin and Eosin
(2) Aldehyde fuchsin
(3) Van Gieson Stain.

## HAEMATOXYLIN AND EOSIN STAIN

1. Section brought to water (i.e. dewaxed and hydrated).
2. Ehrlich's Haematoxylin — 10 minutes
3. "Blueing" in tap water — 5 minutes
4. Differentiate in acid-alcohol — 2 seconds
5. Re-blueing in tap water — 10 minutes
6. Eosin — 1 minute
7. Rinse in distilled water until appropriate amount of eosin remained.
8. Dehydrate and mount (as described).

USE: It is a general purpose stain, staining not only cell nuclei and cytoplasm, but also connective tissue. It distinguishes basophilic materials (blue) from eosinophilic materials (pink).

RESULTS:

| | | |
|---|---|---|
| Nuclei | ---- | blue |
| Collagen | ---- | pink |
| Erythrocytes | - | bright red |
| Muscle | ---- | red |
| Keratin | ---- | red |
| Cytoplasm | ---- | pale pink |
| Fibrin | ---- | pink |

## ALDEHYDE FUCHSIN STAIN

1. Section brought to water.
2. Rinse several times with 95°/₀ alcohol.
3. Aldehyde fuchsin stain, 30 minutes.
4. Rinse off excess stain with 95°/₀ alcohol.
5. Rinse with water.
6. Counterstain with Van Gieson Stain.
7. Dehydrate and mount.

Step No, 6 may be replaced by a light green stain for the background.

USE: It is used as a stain for elastic fibres, and for demonstrating the presence of mucopolysaccharides.

RESULTS:

Elastic fibres ---- deep purple
Mucin, hyaline cartilage ---- purple
Other tissues according to the counter-stain used.

N.B. The same techniques may be used on an oxidised section for demonstrating the presence of Oxytalan fibres (Oxidation: Ozone 10°/₀ for 20 min., at 25°C.)

## VAN GIESON STAIN

1. Section brought to water
2. Weigert's Haematoxylin 10 minutes
3. Rinse in distilled water
4. Van Gieson stain 5 minutes
5. Rinse in distilled or tap water
6. Dehydrate and mount

USE: It may be used either as a general purpose stain or as a special stain for collagen.

RESULTS:

| | | |
|---|---|---|
| Collagen | ---- | red |
| Nuclei | ---- | bluish black |
| Muscle fibres | ---- | yellow |
| Fibrin | ---- | yellow |
| Erythrocytes | ---- | yellow |

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