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CELLULAR KINETICS WITHIN THE MANDIBULAR JOINT*

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INTRODUCTION

The clinical significance and unique features of the temporomandibular joint have brought about a great number and variety of investigations. The orthodontists seek a better understanding of condylar growth to explain the dynamic development of the mandible and compensatory morphologic changes occurring during intermaxillary treatment: while those in pathology find the temporomandibular joint of equal importance in regard to abnormal retardation and acceleration of condylar growth as well as arthritic involvement of the joint structures.

The complexity of these problems have not been clarified through clinical studies because of a lack of basic information on a cellular level. Consequently, it was felt that a detailed investigation into the cellular kinetics of the different components of the

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joint was necessary to provide a basic understanding of the behavior of the mesenchymal cells within the squamosomandibular joint of the rat under normal conditions.

REVIEW OF LITERATURE

Anatomical Considerations of the Mandibular Joint of the Rat

In a strict sense the rat has no temporomandibular joint as the articulation of the mandibular condyle is with the squamosal bone. Sicher (91) has suggested the term squamosomandibular joint to eliminate confusion in the literature with the term temporomandibular joint.

Development

The formation of the squamosomandibular articulation as described by Bhaskar (8) and Cunat et al (24) begins at 17 days insemination age. At this time the condylar cartilage is separated from the condylar blastema followed two days later by the beginning of calcification of the squamosal bone to form a bony plate, the squama. From this plate of bone, the zygomatic process projects outward and forward. The posterior end of the condylar cartilage can be seen ventromedially to the anterior part of the zygomatic process and separated from it by a wide zone of mesenchyme. At 19 days insemination age a wide slit appears within the mesenchyme dividing it into a superior part covering the cranial portion of the joint and an inferior part covering the dorsolateral surface of the condylar cartilage. A second slit develops at 20 days insemination age between the first and the condylar cartilage. The tissue between the two slits becomes the future articular disc.

Cranial Portion of the Joint

Collins, Becks, Simpson and Evans (20) describe the cranial portion of the joint as formed by an elongated groove in the squamosal bone. The long axis of the groove lies in the anteroposterior plane and is directed upward and backward. The fossa is divided into an anterior and posterior part by a rounded eminence which is continuous with the zygomatic process of the squamosal bone. The portion of the fossa posterior to the eminence accommodates the condyle of the mandible when the molars are being used in chewing and the condyle moves anteriorly to the eminence when the incisors are being used in gnawing. The squamosal bone is histologically a membraneous bone covered by fibrocartilage to create the cranial articular surface of the joint. With increasing age this cartilage persists but conforms to the continually changing shape of the condyle (20). Similar findings have been reported in guinea pigs by Öberg (108) and in man by Kerr (48) and by Sicher (90).

Articular Disc

The morphology of the articular disc in the rat is similar to that described for humans by Choukas and Sicher (18). The upper surface of the disc is saddle-shaped, concave anteroposteriorly and markedly convex mediolaterally to adapt to the squamosal bone. The lower surface of the disc is concave in both directions to accommodate the avoid shape of the condyle. The central portion of the disc is considerably thinner than its periphery and composed of a dense, avascular and relatively acellular fibrous tissue (89). At the periphery of the structure the tissue becomes loosely arranged and blends into the adjacent capsule and muscle attachments. Posteriorly, the disc is continuous with a thick layer of loose, vascularized connective tissue arising from the posterior wall of the articular capsule constituting the retrodiscal pad (89). Interspersed between the connective tissue fibers are vessels of various size which not only contribute to the spongy consistency of this particular structure but also constitute the major nutritional source of the joint cavity. These vessels are closely associated with the synovial fringes (6, 28, 40, 62, 67, 79).

Condylar Head

The condylar head presents a surface for articulation with the articular disc of the joint. The anteroposteriorly directed long axis of the condyle is, in the adult rat, approximately twice the length of the short axis. This is in contrast to that observed in man (20). The condylar cartilage is derived from the condylar blastema, a condensation of mesenchymal cells independent of Meckel's cartilage, and then transferred to the mandibular bone as a secondary cartilage (2, 8, 86, 99). The condylar cartilage of the rat, by 25 days of age, has differentiated into five distinct segments. These have been classified by Collins, Becks, Simpson and Evans (20) as the fibrous, embryonic intermediate, vesicular and erosion zones. The outermost layer of the condylar cartilage consists of the fibrous zone under which one observes the embryonic zone, a layer of flat elongated cells having their long axes arranged parallel to the articular surface. The intermediate zone is located beneath the embryonic zone and is characterized by round and enlarged cells separated by an increased amount of matrix. The fourth layer, the vesicular zone, is recognized due to the vacuolization of the chondrocytes which now are large in size, slightly elongated, and perpendicular to the articular surface. The innermost segment, the erosion zone, consists of vacuolated cells in close contact with each other and immediately facing the mandibular bone. With increasing age there is a reduction in the number of cells and actual thickness of all segments described. This occurrence is more pronounced in the vesicular zone (20, 34).

Clinical Consideration Related to the Temporomandibular Joint

The anatomical and functional complexity of the temporomandibular joint has produced a wide spectrum of investigative work.

The problem of condylar growth has been of particular interest among anatomists and orthodontists. In a study of growing rats, Bhaskar (8) found that the condylar cartilage not only serves as a center for growth but contributes by its directional proliferation to the shape of the mandibular ramus. Up to three days of age in rats the condylar growth pattern is mainly backwards, then it changes markedly as indicated by the active apposition within its perichondrium to grow predominantly upward, while the growth in backward and lateral directions continues unchanged. This alteration in inclination of directional growth serves to heighten, as well as, lengthen the mandible. This is in agreement with Charles (17), who concludes from a study using rats that the fibroblastic differentiation into chondroblasts directs the condylar cartilage upward and backward. The mandible is therefore traveling downward and forward. Studies using human embryos have further substantiated the findings that the condylar cartilage is a direct controlling factor of forward mandibular growth. Björk (9) has made more intricate observations in humans by placing metallic implants in the mandibular body of young children in order to follow the events of condylar growth longitudinally. He found that the direction of the ramus varied widely in the sagittal plane among different individuals and the angular region of the mandible was altered accordingly to maintain occlusal harmony.

Even if the shape and general size of the mandible is genetically determined, there is a continuous adaptation to intrinsic and extrinsic factors during growth and function (84). This is of particular importance in regard to the growth mechanism of the condylar cartilage since the embryonic zone, responsible for the oppositional growth, persists throughout life. The existence of these cells might, therefore, at any time open the possibilities of reactivating the growth potential of the mandibular condyle (3, 4, 20). This arrangement would, according to Becks et al (20), allow for remodelling to accommodate chewing habits, muscle pull, and other extrinsic factors which may influence the development of the temporomandibular articulation (17, 102). Further, the potentiality for condylar growth has stimulated Baume et al (5) and Pruzansky (76) to develop therapeutic measures in the treatment of Pierre Robin's syndrome.

Baume and Derichsweiler (3, 4) questioned whether or not the condylar growth center in adult monkeys is responsive to orthodontic therapy. By introducing inclined biteplanes, the mandible was displaced in a forward direction. On examination afterwards, the condylar head was found to be prolonged and bilobed in shape as a compensatory growth response to the mandibular placement. Sicher (91), however, disagrees with Baume and Derichsweiler's conclusive statement that their observations were a response of condylar growth. He considers their results a normal expression of a joint to regain proper and functional relationships.

In studying other articulations, the histology and cytology of the epiphyseal plate are well documented, while the detailed mechanism which regulates normal, longitudinal growth is practically unknown (65). Clark and Clark (19) found that the perichondral cells possess specific potentialities to form cartilage; thus, they are essential for endochondral growth, since division by mitosis in differentiated cartilage is rare. Clinical studies by Shands (87), however, reveal no definite evidence of perichondrium covering the articular surface of adult hyaline cartilage in dogs, while similar studies of the condylar cartilage have demonstrated a proliferative zone favoring mandibular growth (3, 4, 41, 91) Sicher (89) further found the condylar cartilage holding a unique position as the connective tissue covering enables the cartilage to increase in thickness not only by interstitial but also by appositional growth.

In spite of the fact that the temporomandibular joint demonstrates this unique adaptability (91), pathologic changes do appear. A number of clinical and histologic observations have documented degenerative alterations, while very few studies deal with the reparative capacity of the temporomandibular joint. Blackwood (12) and Kerr (48) have described the central portion of the meniscus and articular surface of the condylar cartilage to be the first sites of arthritic breakdown. The meniscal involvement has mainly been associated with the poor vascular supply to the midportion, which by many investigators is thought to jeopardize the maintenance of collagen fibers (48, 62, 98); Choukas and Sicher (18) and Sicher (89) believe that this avascular arrangement is a physiologic expression of a pressure-bearing area. Clinically, but without nexus, it has been observed by Summa (98) that the incidence of meniscal perforations is particularly high in edentulous patients.

Blackwood (12) and Kerr (48) further stated that following the destruction of the protective fibrous layer of the condyle, the condylar cartilage undergoes a hyalinization and an atypical chondrogenic proliferation resulting in a thickened, rough-surfaced articular cartilage. The changes observed in the articular fossa, on the other hand, appeared to be secondary to those of the articular disc and condylar cartilage (21).

Studies of DNA Synthesis and Cell Kinetics with Tritiated Thymidine

The use of radioactive isotopes as tracers in biological research permits the study of the metabolism of specific substances within the living organism. One such compound, tritium labeled thymidine, is incorporated into deoxyribonucleic acid (DNA) whenever chromosomes are duplicated prior to cell division (1, 37, 45, 75, 80, 85). Therefore, by introducing tritiated thymidine into an animal, certain nuclei become labeled and retain this labeling as long as the cells survive, except for dilution of the isotope following mitosis. Consequently, the progress of these labeled cells may be followed with time radioautographically. This methodology is based upon many assumptions and a great number of investigations have been undertaken to confine the validity of its technical and psysiological applications (7, 22, 23, 32, 33, 39, 42, 44, 46, 50, 51, 54, 57, 63, 64, 72, 74, 75, 77, 78, 81, 83, 94, 97, 100, 101, 104).

Tritiated thymidine not only provides information about the mechanism of DNA synthesis but also makes it possible to study chronological events within the cell cycle. In order to proceed from one division to the next, the proliferating cell has to pass through different phases of the cell cycle, which have been divided into four periods: S, G_2, M, and G_1 stage.

The S phase represents that part of the interphase when the deoxyribonucleic acid is synthesized. Recent investigations disclosed the duration of the S phase to be fairly constant, lasting from 6—8 hours in many mammalian cell populations (16, 47, 49, 73, 74, 105, 106). Sherman, Quastler, and Winberg (88) have reported, however, that the ear epidermis demonstrates an S phase of 30 hours which seems to disperse the hypothesis of its constancy. This has also been the subject of a paper by Bresciani (15) who found that ovarian hormones accelerate the process of DNA synthesis in mammary cells of mice. Along the same lines of thought Edward and Klein (30) have postulated that even local factors such as cell metabolites do have an influence on DNA synthesis of adjacent cells. The mechanisms behind these observations are still speculative.

The S phase is followed by a brief period, the premitotic gap or G_2 phase, intervening between completion of DNA synthesis and the onset of morphologically discernible mitosis. Defendi and Manson (27) and Koburg and Maurer (49) have found that the G_2 phase is fairly uniform in duration, ranging from 1—4 hours in different cell populations. Certain cells in mouse epidermis are exceptional, however, as they remain in G_2 for an indefinite period of time (38, 70). Gelfant (38) has interpreted this behavior as a way to create a reservoir of cells which immediately undergo mitosis, while Pelc (70, 71) suggests that incorporation of H^3-thymidine is possible in some differentiated cells as a process of renewal and exchange of genetic material, DNA, without subsequent mitosis. The cellular behavior described by Pelc has further been attributed to the ameloblast by Stallard, Diab, and Zander

(96). In the study of cellular kinetics of osteogenic tissue, Owen (69) has made similar observations that some osteoblasts retained their labels for several days following injection and became osteocytes without division.

Following the G_2 phase the cells proceed into the mitotic, or M, phase. Its duration differs between cell types but generally falls in the range of 40 to 70 minutes, although durations up to several hours have been reported for some tissues (7, 38, 45, 52, 58, 74, 85, 101, 105, 106).

The daughter cells arising from the labeled mitoses enter the postmitotic or G_1 phase, where some immediately recommence the cell cycle while others differentiate to perform the functional activities characteristic to the particular cell type. These cellular processes in G_1 are continuously influenced by environmental factors such as pH and nutritional conditions, which are considered to be of great significance in regulating the incidence of mitosis and proliferation rate, both within and between cell populations (15, 26, 105, 106). Consequently, the duration of the G_1 phase is highly variable as well as tissue specific. Average of 5 hours within the growing liver has been reported by Post and Hoffman (74), while corresponding values for the periosteum of the rat equals 104 hours (105, 106).

Studies of Articular Cartilage and Endochondral Ossification with Tritiated Thymidine

In 1960 Messier and Leblond (63) presented the first preliminary investigation of cell migration within the epiphyseal plate using H^3-thymidine. Kember (47) similarly analyzed the cellular behavior of the metaphyseal plate in growing rat tibiae and observed that the S and M phases averaged 8.5 hours and 41 minutes respectively within the cell population. From the rate of cell division, Kember also estimated the daily bone growth to approximately 125 microns. Young (105, 107) expanded Kember's work to a comparative study of the metaphysis, endosteum and periosteum of the rat tibiae. He reported an S phase of 8 hours in all areas while the generation time averaged 36, 57 and 114 hours within respective locations. The differences in generation times were ascribed to the variations in the lengths of the G_1 phase, since the G_2 plus M interval varied only slightly.

Articular Cartilage

In studies of immature articular cartilage Mankin (58) observed in rabbits two zones of cellular proliferation. The layer subjacent to the articular surface increases the thickness of the articular surface and possibly compensates for attrition of the joint surfaces, while the deeper layer contributes to the growth of the ossific nucleus of the epiphysis. With increasing age of the articular cartilage, Mankin (61) noted a gradual cessation of cell proliferation beginning in the superficial zone. As the animal reached adult age no further radioautographic or histologic evidence of growth was seen in any of the two layers.

Effect of Hormones

Hormone experiments have also been performed to elucidate the effect upon the cell cycle of osteoprogenitor as well as chondroprogenitor cells. Simmons (93) indicates that the primary effect of estrogen or its metabolites occurs at 6 to 12 hours after H^3-thymidine administration and acts to stimulate the onset of undifferentiated bone marrow cells to form osteoblasts. The rate suggests that the generation time for these cells is briefer in estrogen treated groups of mice. Bois, Belanger, and LeBuis (14) investigated the effect of growth hormone and found that hypophysectomized rats had a smaller epiphyseal plate with a decreased mitotic activity. By administering growth hormone, however, the normal incidence of mitotic activity was restored.

Condylar Cartilage

Radioautographic information on the condylar cartilage of the mandible is at present limited to several abstracts appearing in the literature. Preliminary studies by Blackwood (10, 11), Dale et al (25), Folke (34), and Folke and Stallard (35, 36) have revealed that the embryonic (transitional) zone constitutes the only important layer of cell proliferation within the condylar cartilage. By injecting tritiated thymidine it has also been observed that the chondrocytes eminate from the embryonic zone and migrate toward the medullary cavity to be found in different cell layers of the cartilage by 96 hours (10, 11, 13, 25, 36).

PRESENT INVESTIGATION

MATERIALS AND METHODS

This investigation is an autoradiographic study of the cellular kinetics within different structures of the squamosomandibular joint of the rat. In order to follow the intricacies of DNA duplication and subsequent cellular division, tritiated thymidine was utilized. The animals were sacrificed at various time intervals after the injection of the isotope to determine the individual cell cycle, differentiation and migration of cells which originated within the embryonic zone. In addition, the individual labeled indices of the articular cartilage, articular disc and condylar cartilage were observed and recorded.

Animals

Fifty-eight male Sprague-Dawley rats were utilized in this investigation. They were all four weeks old at the time of H^3-thymidine injection and their weight averaged 117.4 grams. The animals were maintained on a balanced diet and under standard conditions of lighting (12 hours light, 12 hours darkness) throughout the entire experimental period permitting normal growth and development.

Experimental Intervals

In order to determine and compare labeled indices and the relative rates of cellular division, all animals received at the same hour (10:30 a. m.) an intraperitoneal injection of H^3-thymidine, in a dosage of 1 uc per gram of body weight. The animals were then sacrificed in pairs at 1, 1½ hours, at one-hour intervals from 2 to 12 hours, at 2-hour intervals from 12 to 24 hours, at 12 hour intervals from 36 to 108 hours, and at 132, 156, and 180 hours after injection. From this horizontal approach it was possible to determine the labeled index at different time points and to calculate the length of the various stages of the cell cycle, follow migration, differentiation, and ultimate death and resorption of the chondrocyte within the condylar cartilage.

Isotope

A DNA precursor, tritiated thymidine, was utilized to label cells and follow their cellular kinetics. H^3-thymidine was administered by intraperitoneal injection in a dosage of 1 uc per gram of body weight. The isotope* had a specific activity of 6.76 curies per millimole and was supplied in sterile aqueous solution.

Tissue Preparation

Upon sacrifice by decapitation the heads were skinned and immediately fixed in Lavdowsky's solution for 72 hours. Decalcification of hard tissues was accomplished within 7 days by a mixture of 20 % formic acid and Winn-3000, an ammoniated salt of a sulfonated resin. The Winn-3000, acting as an ion exchange resin, aided in the rapid decalcification of the tissues. Cellular details of both the hard and soft tissues were satisfactorily preserved by this method (29). Following demineralization, the specimens were returned to 80 % alcohol, for 48 hours in order to remove the formic acid. After embedding, sagittal as well as transverse sections were cut at 3 microns through the squamosomandibular joint. Routin deparaffinization and hydration methods were followed prior to radioautographic and staining procedures.

Radioautography

On the completion of tissue preparation the slides were dipped, in total darkness, into bubble-free Kodak NTB-3 emulsion at 40°C, and left to dry completely for two hours at 20°C. The slides were then exposed in dry atmosphere for 21 days at 4°C. The preparations were stained either by the Feulgen technique prior to radioautography, or by Harris' Hematoxylin after exposure and development.

Analysis

The radioautographic analysis was performed separately for the articular cartilage, articular disc and the condylar cartilage of the squamosomandibular joint.

Slides to be studied were chosen at random, and a total of 30 sections were studied from each time point. The autoradiograms were examined under oil immersion to determine the number of labeled as well as unlabeled cells and mitotic figures. Eight or

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* New England Nuclear Corporation, Boston, Massachusetts.

more exposed silvergrains over the nucleus classified the cell as labeled and counted accordingly. Sections were disqualified, however, if the background of unrelated exposed silvergrains exceeded 2 grains per 100 μ^2.

The number of labeled cells per 1000 counted cells within each cell population were counted and will hereafter be referred to as the labeling index (Chart 1 and 2). A total number of at least 100 metaphases (Fig. 4 a, b, c and 6 a, b) were also counted within the embryonic zone of the condylar cartilage to determine the percentage of labeled metaphases at various times of sacrifice (Chart 3).

From these graphs it is possible 1) to determine the labeled index within each cell population studied and compare DNA synthesis with subsequent mitosis and 2) to estimate the duration of the DNA synthesis stage (S_{DNA}), the post-synthesis stage (G_2), the mitotic stage (M), and the postmitotic stage (G_1) of the cells within the embryonic zone. These four phases constitute the progenitor cell cycle, which has a duration period equal to the average time that elapses between successive divisions of progenitor cells (Chart 4).

The length of time from the administration of tritiated thymidine to the first appearance of a labeled prophase represents the minimal duration of the G_2 phase. Similarly, the period elapsing from the time of injection to the first labeled telophase indicates the minimal duration of the G_2 and mitotic phases. The mean S phase was ascertained by measuring on the curve of labeled metaphases (Chart 3) the time intervening between the 50 % levels of the ascending and the descending limbs.

When the average length of DNA synthesis phase is known, as well as the labeling index at one hour after H^3-thymidine injection, the *average* duration of the progenitor cycle (generation time) can be calculated as suggested by Quastler and Sherman (78) in the following manner:

$$\frac{n}{N} = \frac{t}{T}$$

n = Number of labeled cells at 1 hour after H^3-thymidine injection.

N = Number of all progenitor cells counted.

t = Mean duration of S-phase.
T = Mean duration of whole progenitor cycle (generation time).

Thus: $G_1 = T - (G_2 + M + S)$

To study the migration of labeled cells within the condylar cartilage a central portion of the cartilage measuring 360 microns in thickness was chosen. This area was subdivided into three zones of 120 × 300 microns using a squared eyepiece graticule and the location and number of labeled cells were plotted (Chart 5).

RESULTS

The observations and results of this investigation will be divided into three sections due to the differences in morphology and cellular behavior between the fibrocartilage of the squamosal bone, the articular disc, and the condylar cartilage. The results are recorded in tables I—VI and graphically summarized in charts 1—5.

The Fibrocartilage of the Squamosal Bone

This structure covers the squamosal bone and constitutes the articular fossa of the mandibular joint. Several different tissue layers are distinguished within the fossa. The outermost zone, facing the joint cavity, consists of 1—4 rows of flattened cells interspersed between collagen fibers which all have their long axis parallel to the surface (Fig. 1 and 2). Beneath this layer there is a zone of mesenchymal cells which gradually merge into a narrow band of hyaline-like cartilage. This structural arrangement is similar to that seen in the condylar cartilage except for the fact that the cartilage of the fossa is devoid of an erosion zone and therefore directly attached to the compact part of the squamosal bone.

The autoradiograms indicate that the cellular activity is localized primarily within the mesenchymal cells, while the surface layer exhibits very few labeled cells. The labeling index of the fibrocartilage as a whole was found to be 21.6 at one hour and rose to 44.4 at 10 hours after injection of H^3-thymidine. Within the experimental time, it was observed that the labeled mesenchymal cells differentiate into chondrocytes, but never become a cellular component of the underlying compact bone. Some cells also migrated toward the articular surface and were subsequently lost, as substantiated by the drop in the labeling index to 15.4 at 18 hours after injection (Table I and Chart 1).

Table I. *Distribution of labeled cells in the fibrocartilage of the squamosal bone.*

Time after injection of H^3-Thymidine	Total number of counted cells	Number of labeled cells	Per mill labeled cells
1 Hour	7353	159	21.6
2 Hours	7362	166	22.5
3 Hours	7525	195	25.9
4 Hours	7013	171	24.4
5 Hours	7442	213	28.6
6 Hours	7026	221	31.5
7 Hours	7029	261	37.1
8 Hours	7057	284	40.2
9 Hours	7027	301	42.8
10 Hours	7123	316	44.4
11 Hours	7062	225	31.9
12 Hours	7106	129	18.2
14 Hours	7045	142	20.2
16 Hours	7129	113	15.9
18 Hours	7069	109	15.4

Articular Disc

The main constituent of the biconcave portion of the articular disc are the curly, dense collagen bundles. Elastic fibers are also running parallel to the articular surface. The cells are round or oval in shape and scattered widely throughout the collagen fibers (Fig. 1 and 2). The labeling index of this cell population is only 1.7 at one hour and rises to 4.4 at 11 hours after injection (Table II and Chart 1). In the anterior and posterior part of the disc, there is a looser arrangement of connective tissue fibers in association with adipose tissue and a rich vascular supply. The labeling index was not calcutated in these areas, however, a great number of labeled cells were observed.

Condylar Cartilage

The identification of the different zones within the condylar cartilage is simplified by the alteration in cell density and the different character of the nuclei (Fig. 3). The nuclei are nearly flat at the articular surface and gradually increase in size and become spherical toward the erosion zone.

The fibrous zone of the condylar cartilage is an avascular connective tissue composed of 3—6 layers of flattened cells arranged in a fashion similar to that seen in the articular fossa (Fig. 2 and 3). The labeling index was found to be 12.1 at one hour and increased to 33.8 at 11 hours after injection of H^3-thymidine. By 18 hours the labeling index had decreased to 15.0, which may be indicative of cellular attrition on the articular surface (Table III and Chart 1).

Table II. *Distribution of labeled cells in the articular disc of the squamoso-mandibular joint.*

Time after injection of H^3-Thymidine	Total number of counted cells	Number of labeled cells	Per mill labeled cells
1 Hour	4133	7	1.7
2 Hours	4251	8	1.9
3 Hours	3612	8	2.2
4 Hours	4996	19	3.8
5 Hours	4080	11	2.7
6 Hours	4869	16	3.3
7 Hours	4282	14	3.3
8 Hours	4384	17	3.9
9 Hours	4333	17	3.9
10 Hours	5016	21	4.2
11 Hours	4132	18	4.4
12 Hours	3151	11	3.5
14 Hours	5087	21	4.1
16 Hours	4321	18	4.2
18 Hours	4053	11	2.7

Table III. *Distribution of labeled cells in the fibrous layer of the condylar cartilage.*

Time after injection of H^3-Thymidine	Total number of counted cells	Number of labeled cells	Per mill labeled cells
1 Hour	5061	61	12.1
2 Hours	5031	65	12.9
3 Hours	5069	81	16.0
4 Hours	5023	78	15.5
5 Hours	5002	82	16.4
6 Hours	5143	97	18.9
7 Hours	5037	120	23.8
8 Hours	5078	147	28.9
9 Hours	5311	156	29.4
10 Hours	5117	164	32.1
11 Hours	5054	171	33.8
12 Hours	5187	103	19.9
14 Hours	5066	79	15.6
16 Hours	5029	81	16.1
18 Hours	5071	76	15.0

The cells of the embryonic zone located beneath the fibrous layer (Fig. 3) were studied in detail during the first 24 hours after H^3-thymidine injection, to determine the labeling index with time (Fig. 5). These cells were densely packed and demonstrated the highest cellular activity of the tissues studied as indicated by the labeling index of 65.8 at one hour after injection. By 11 hours the index reached its peak of 109.2 and then slowly decreased to a value of 88.7 at 24 hours after injection (Table IV and Chart 2).

The first labeled prophase was observed at 1½ hours and the first labeled telophase at 3 hours after injection. The percentage of labeled mitotic figures reached a plateau at 3 hours (86.8 %) and remained there until 7 hours (86.0 %) before dropping off rapidly. The labeled mitotic index reached its peak (94.0 %) at 5 hours after injection. These results were summarized in Table V and Chart 3. From these data it is possible to trace the progression of labeled cells from the DNA synthesis stage through mitosis and thereby estimate the length of the synthesis stage, G_2 stage and the mitotic stage of the cells within the embryonic zone.

Since the first labeled prophase was observed at 1½ hours but not at 1 hour following injection of tritiated thymidine, the minimum G_2 period was between 1 and 1½ hours in duration. The first labeled telophase was seen at 3 hours (Fig. 7) indicating that the minimal duration of the combined G_2 and mitotic phase is 3 hours or less. The mitotic phase, therefore, has an average duration of approximately 1½ hours (Chart 4). The S phase was calculated by taking the time interval between the 50 % labeled mitotic figures of the ascending and descending limbs as indicated in Chart 3. This gives a value of 7 hours for the S phase (Chart 4). The progenitor cycle time is then calculated according to the relationship earlier described and the average generation time (T) was found to be 100 hours. The post-mitotic gap (G_1) is then obtained by subtracting the sum of the times for G_2, mitosis and DNA synthesis from the total replication time. This revealed for the cells of the embryonic zone an average duration of 90 hours (Chart 4).

In studying the effect of time upon labeled cell distribution it was observed that the greatest number of labeled cells was found in the central portion of the embryonic zone at 1 hour after injection. Only a few labeled cells were present in the fibrous and in-

Table IV. *Distribution of labeled cells in the embryonic zone of the condylar cartilage.*

Time after injection of H^3-Thymidine	Total number of counted cells	Number of labeled cells	Per mill labeled cells
1 Hour	20.000	1315	65.8
2 Hours	20.000	1370	68.5
3 Hours	20.000	1463	73.2
4 Hours	20.000	1452	72.6
5 Hours	20.000	1551	77.6
6 Hours	20.000	1648	82.4
7 Hours	20.000	1874	93.7
8 Hours	20.000	1967	98.4
9 Hours	20.000	2038	101.9
10 Hours	20.000	2042	102.1
11 Hours	20.000	2184	109.2
12 Hours	20.000	1922	96.1
14 Hours	20.000	2015	100.8
16 Hours	20.000	1841	92.1
18 Hours	20.000	1899	95.0
20 Hours	20.000	1824	91.2
22 Hours	20.000	1843	92.2
24 Hours	20.000	1774	88.7

Table V. *Distribution of labeled mitotic figures with time after injection of H^3-thymidine.*

Hours after injection	Number of mitotic figures	Number of labeled mitotic figures	Percentage labeled mitotic figures
1 Hour	132	0	0
1.5 Hours	147	6	4.1
2 Hours	134	61	45.5
3 Hours	121	105	86.8
4 Hours	138	121	87.7
5 Hours	116	109	94.0
6 Hours	122	107	87.7
7 Hours	129	111	86.0
8 Hours	102	67	65.7
9 Hours	139	68	48.9
10 Hours	126	46	36.5
11 Hours	130	37	28.5
12 Hours	128	17	13.3

termediate and vesicular zones and at 96 hours after injection the first labeled cell reached the erosion zone. At 156 hours after injection there was a rather uniform distribution of labeled cells within the vesicular zone and by 180 hours the labeled cells were distributed equally throughout the three segments studied (Table VI and Chart 5).

Table VI. *Distribution and migration of labeled cells within the condylar cartilage.*

Microns from articular surface	Hours after injection of H^3-Thymidine																			
	36		48		60		72		84		96		108		132		156		180	
	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%	No. cells	%
0—120	932	73.4	579	61.9	662	59.5	653	48.2	474	52.1	424	42.7	533	39.4	547	38.2	496	37.3	581	32.4
120—240	337	26.6	356	38.1	451	40.5	648	47.9	357	39.3	465	46.8	567	41.9	599	41.8	461	34.6	648	36.2
240—360	0	0.0	0	0.0	0	0.0	53	3.9	78	8.6	105	10.5	252	18.7	286	20.0	374	28.1	563	31.4
	1269	100.0	935	100.0	1113	100.0	1354	100.0	909	100.0	994	100.0	1352	100.0	1432	100.0	1331	100.0	1792	100.0

The distance from the embryonic zone to the edge of the cartilage plate adjacent to the erosion zone was approximately 240 microns. The first labeled cell to migrate this entire distance was observed at 96 hours, indicating maximal daily rate of condylar growth of approximately 60 microns. Beyond 24 hours, after injection of tritiated thymidine, a rapid decline was observed in the number of labeled cells within the embryonic zone. This drop leveled off at 96 hours and a plateau persisted until 180 hours, after which time a second decrease was noted (Table VI and Chart 5). During migration the number of silvergrains over each labeled cell remained the same.

DISCUSSION

The application of H^3-thymidine autoradiography adds a third dimension to the histological interpretation of cellular kinetics. This technique, the validity of which has been referenced in the review of literature, facilitates the examination of specific cell populations to determine their rates of proliferation, migration and differentiation. Calculating the progenitor cell cycle is probably of most importance, since this reflects cell renewal and thus regulates tissue expansion or turnover.

Preliminary studies have revealed that the cells in the embryonic zone of the condylar cartilage are of the greatest importance as a progenitor population; therefore, attempts were made in this study to determine their cell cycle. Several investigations (16, 22, 43, 49, 66, 74, 78, 95, 105, 107) have dealt with the cell cycle per se and generally agreed that the precision of time measurement obtained should be accepted with some caution due to mistaken classification of cells and temporal cellular variability when comparing different animals. It is difficult to classify the cells of the embryonic zone into progenitor cells and recently differentiated, nondividing cells as they are morphologically similar. Such shortcomings do effect studies of cellular behavior which is in agreement with Cameron and Greulich (16) and Leblond, Greulich and Pereira (53). Furthermore, subsequent mitoses dilute the thymidine labeled material, thereby decreasing the number of silvergrains overlying each nucleus making determination of labeled cells with time inaccurate. Misinterpretations of labeled cells would therefore immediately alter the labeling index. It should also be emphasized that increasing thickness of the tissue sections distorts the resolution and results in adverse effects upon the mitotic and labeling indices due to the short range of beta particles from each tritium labeled cell. Disregarding such sources of error leads to incompatible conclusions within as well as between studies.

The duration of the S phase of the embryonic cells was approximately 7 hours and was obtained by calculating the time interval between the 50 % levels of the labeled mitotic curve (Chart 3). This curve is theoretically based on the relationship between labeled and unlabeled metaphases (16, 49, 55, 78, 95, 105, 106). Serious attempts were made to consider only metaphases and early anaphases (Fig. 4 a, b, 6 a and b) but the 100 % level of the mitotic index was never reached, indicative of the fact that certain of the labeled cells may remain in G_2 or simply escape registration in their metaphase. This suggests that the labeled mitotic cells reach the 50 % level at an earlier time point on the ascending curve and correspondingly later on the descending limb resulting in an S phase between 7 and 7.5 hours. This duration of S is in agreement with those obtained from other cell populations (16, 38, 47, 49, 58, 74, 96, 101, 105, 106) suggesting that this phase of the cell cycle is relatively constant regardless of cell type. The calculation of the G_2 phase was based upon the observation of the first labeled prophase which revealed a minimum average duration of 1 to 1.5 hours. This estimation coincides with earlier studies by Young (105, 106) of osteogenetic tissues. An improvement of the methodology to calculate G_2 has been suggested by Post and Hoffman (74) by plotting a labeled prophase curve and determining the average G_2 from the time interval between the administration of the tritiated thymidine and the time of 50 % of prophase nuclear labeling. This would, in addition, provide a theoretically better approach to the study of cellular behavior in G_2, since environmental influences, as suggested by Gelfant (38), may alter its duration and that renewal of genetic material may take place without subsequent cellular division as postulated by Pelc (70), Owen (68), and Stallard et al (96). An accurate labeled prophase curve was difficult to obtain due to superimposition of silvergrains on nuclear morphology and thus is not included in the present investigation.

Labeled telophases were, however, easily recognized and the first appeared at 3 hours following H^3-thymidine injection indicating the minimum duration of G_2 + M was 2—3 hours. Since the G_2 phase was calculated to 1.5 hours, the M phase subsequently averages 1 to 1.5 hours. These findings are consistent with those for the metaphysis and endosteum as reported by Young (105, 106). Kember (47) reached a similar calculation of the G_2 phase but found the M phase to be of approximately 41 minutes within the metaphysis of growing rat tibiae. The discrepancy in these M values seems insignificant since numerous studies of other cell systems have revealed that this phase is very constant, once initiated, and falls within the range of 30 minutes to 2 hours. This variation would have very little influence on the total cell generation time to account for the differences among cell populations.

The G_1 phase, on the other hand, is the most potential factor in regulating the cell generation time and its duration is extremely difficult to approach due to the many events taking place in the G_1 compartment (Chart 4). Based on a 7 hour S period and an average labeling index at 1 hour after H^3-thymidine injection, the cell generation time was estimated to be about 100 hours within the embryonic zone of the condylar cartilage. This calculation of the generation time is based upon the original formula by Quastler and Sherman (78) and therefore has an inbuilt error, as we cannot assume that all cells within the embryonic zone have the same capacity to divide. In order to decrease this source of error all cells immediately adjacent to the intermediate zone were excluded from consideration as they represent primarily differentiating and nondividing cells of the embryonic zone. This was evidenced by the simultaneous entrapment of labeled daughter cells within the intercellular matrix of the intermediate zone. The embryonic zone represents therefore an inhomogenous renewing cell population with very few cells having a generation time equal to the predicted value due to the observed variations in the G_1 phase.

Even if more precise values become available through technical improvements, the present methodology has definite merits in comparing the average cell generation time between different cell populations. According to Young (105, 106), the generation time of the metaphyseal, endosteal and periosteal cells in the growing rat tibiae are 36, 62, 133 hours. Cameron and Greulich (16) reported similar variations within the alimentary tract of mice ranging from 181 hours in the esophagus to 16.7 hours in the lower ileum, which are in agreement with earlier observations by Edwards and Klein (30), Lesher, Fry, and Kohn (56), Lipkin and Quastler (58), and Quastler and Sherman (78). The main point of interest in these findings is the great individuality in generation time between different cell systems, since the cell cycle governs tissue renewal time. This variation depends on the functional demand placed on each cell and the 100 hour average generation time of the mesenchymal cells within the embryonic zone of the condylar cartilage must, therefore, fulfil the needs of condylar growth of the mandible to parallel the development of associated facial bones within the four week old rat. In this respect, however, it is important to realize the intimate coordination between cell division and cell differentiation to maintain condylar growth. Consequently, an equilibrium of cell proliferation can be controlled by the balance between the percentage of cells differentiating into chondrocytes and the velocity of the progenitor cell cycle.

In the context that endocrine, local or general systemic factors may promote or inhibit mitotic activity and cell differentiation the embryonic zone of the condylar cartilage is of great interest as it persists throughout life; thus maintaining a regulatory influence on condylar growth thereby influencing the length of the mandible as well as the height of the ramus. Bhaskar (8), Björk (9), Folke (34), and Folke and Stallard (35) have placed considerable emphasis on this developmental correlation of directional growth within the condylar cartilage. This is controlled by the differential rate of cellular division within the embryonic zone only as demonstrated by Folke (34), and Folke and Stallard (35), since there is almost complete absence of labeled cells in underlying cellular compartments.

The principle mechanism of condylar cartilage increase is by appositional proliferation in the embryonic zone since no interstitial growth was observed as suggested by Sicher (89). Some binucleated, chromatin dense cells were found in the intermediate zone of the condylar cartilage suggesting a process of amitosis. Elliott (31) has discussed this form of proliferative activity within articular cartilage to a great extent and believes this is a vital process increasing with age, while Crelin (21) does not believe such division takes place within symphyseal cartilage. The events in the condylar cartilage did not support the hypothesis of amitotic division, since perfect timing of H^3-thymidine injection demonstrated that the "amitotic" cells originate in the embryonic zone and undergo a normal mitotic process, with slightly delayed cytoplasmic separation (Fig. 4 d). Before entering the vesicular zone both labeled cells were completely separated from each other. They later appeared at the erosion zone without further decrease in grain count over each well-defined nucleus, suggesting a continuous G_1 phase upon separation from the embryonic and immediate zones (Fig. 8 a and 8 b). Some labeled nuclei, however, demonstrated comparatively no reduction of silver grains which leaves the question open whether some chondroprogenitor cells differentiate into chondroblasts during the G_2 phase and thus avoid subsequent cell division.

By following these labeled cells it was further possible to calculate the velocity of cell migration from the embryonic zone to the erosion zone. This width averages 240 microns in the midportion of the condylar head and the first labeled cell traveled this distance in 96 hours. An even distribution of labeled cells was observed throughout the vesicular zone at 156 hours (Chart 5) suggesting an average condylar growth of 40—60 microns a day. This is in close agreement with Blackwood's findings (13) within the condylar cartilage and comparatively less than one half of the migration rate within the proximal epiphyseal plate of the tibia as estimated by Kember (47) for rats in the same developmental stage.

The average life span of the chondrocytes was calculated to be about 5—7 days which agrees with earlier findings by Blackwood (11, 13). The life expectancy of these cells becomes conceivably longer with age as the cell proliferation decreases within the embryonic zone accompanied by lesser resorptive activity at the cartilage bone junction.

Upon completion of the mitotic phase there is a rapid differentiation of primarily chondrocytes in the embryonic zone since a decrease of labeled cells was recorded within 24 hours after H^3-thymidine injection. By 72 hours approximately 50 % of the labeled cells were present in cell layers beneath the embryonic zone. Half of the chondroprogenitor cells stay behind to secure the continued growth potentiality of the condylar cartilage (Table VI and Chart 5).

Analyzing the labeling indices of the fibrocartilage of the squamosal bone, the articular disc, the fibrous and embryonic zones of the condylar cartilage one finds an obvious difference in cellular proliferation (Chart 1 and 2). The curves of the fibrocartilage of the squamosal bone and the fibrous zone of the condylar cartilage almost parallel each other in time, while the discrepancy in labeling indices is interpreted as an expression of increased functional load upon the cell population in the former structure to replace not only cells lost through articular attrition, but also to provide chondroprogenitor cells to maintain the cartilage.

The functional significance of the cells in the fibrous zone of the condylar cartilage is to maintain the articular surface only. This is obviously an active process as demonstrated by the labeling index curve. The comparatively high peak obtained at 11 hours after H^3-thymidine injection is beyond the calculated number of labeled cells following one complete cell cycle suggesting that some of these cells are derived from the embryonic zone. The cellular migration suggested is contrary to Dale et al (25), and Blackwood (13) as they found the fibrous and the embryonic zones being two separate compartments without any interchange of cells.

The two different layers described by Mankin (59, 60, 61) in the articular cartilage of the distal femur have apparently different functional purposes. The one subjacent to the articular surface contributes to its growth, while the deeper placed zone is related to the expansion of the epiphyseal nucleus. This is in agreement with studies by Silberberg et al (92) that differentiated chondrocytes within the deeper zone would not migrate towards the surface layer and assume a simpler cellular organization. These observations are not directly applicable to the condylar cartilage of the mandible since the embryonic zone consists morphologically of mesenchymal cells with more pronounced potentiality for cellular modulation suggesting minor participation in the maintenance of the articular surface as well as condylar growth.

The establishment of this functional arrangement has clinical significance. It is important to know whether the fibrous zone can be reestablished following destruction as in the case of osteoarthritic involvement (12, 48) or whether the absence of this fibrous protective layer has any effect upon the cell behavior within the embryonic zone and subsequently condylar growth.

The results in this investigation further suggest that there is a renewal of the surface cells in the fibrocartilage of the squamosal bone and fibrous layer of the condyle as indicated by the rapid drop in labeling indices beyond 11 hours after injection. Such a cell turnover has earlier been questioned in other joints by Mankin (59, 60, 61) and Silberberg et al (92). In substantiating their findings one would expect to see a plateau instead of a decline of the labeling index immediately following the peak value. The latter was observed in this study suggesting that cells are eliminated at the articular surfaces by a process of rapid cell integration rather than simple sloughing of dead cells, since cell fragments were rarely found in the synovial cavities.

The cells within the biconcave portion of the articular disc were widely scattered between the curly collagen fibers and demonstrated the lowest labeling index of the joint structure studied (Chart 1 and Table II). This arrangement is certainly indicative of the fact that this structure is maintained as long as the collagen fibers are intact since the few cells particularly in the central portion of the disc are insignificant in number to bring about a repair of a disc perforation. This lack of reconstructive power has been related by Summa (98) and others to the absence of blood supply, while this investigation suggests that the cellular behavior of the articular disc is organ specific since these cells have the same access to nutrition as those of other more proliferative avascular structures of the joint. The anterior and posterior portion of the disc demonstrated, however, an abrupt increase in labeling index proposing these sites to be developmental and functional adaptation zones for the articular disc. The abundance of blood supply in the latter areas as described by Bernick (6) might be in direct context with the mitotic activity but it is equally important to point out that their anatomical arrangement as well as functional tasks are not comparable to those of the central portion of the articular disc.

SUMMARY

Fifty-eight Sprague-Dawley rats were injected with tritiated thymidine at four weeks of age and sacrificed at various intervals up to 180 hours after injection to investigate changes in the cell behavior with time in the different structures of the mandibular joint. The tissues were processed for histologic and radioautographic analysis, and the following conclusions were drawn:

1. A definite difference in DNA duplication between cell populations within the squamosomandibular joint was demonstrated. A greater labeling index in the embryonic zone was observed, less pronounced in the fibrocartilage of the fossa and the fibrous zone of the condylar cartilage, and almost negligible in the biconcave portion of the articular disc.
2. The cells within the embryonic zone revealed a cell cycle of 100 hours average duration. The S_1, G_2, M and G_1 phases average 7, 1½, 1½ and 90 hours respectively. After division approximately 50 % of the cells remained in the progenitor pool to maintain the reserve cell population while the remaining cells specialize and become chondroblasts.

3. Following differentiation, the labeled chondrocytes retain their labeling suggesting a continuous G_1 phase during their migration within the condylar cartilage. These cells are finally released into the medullary cavity after an average lifespan of 5 to 7 days.
4. The renewal rate of cells in the articular zone of the condylar cartilage is comparatively low and apparently matched to maintain the articular surface and not associated with condylar growth. The labeled cells are mainly derived within the articular layer except a small contribution from the embryonic zone.
5. The labeling indices of the fibrocartilage of the fossa and the fibrous zone of the condylar cartilage were in evidence of cell renewal at their articular surfaces.

RÉSUMÉ

CINÉTIQUE CELLULAIRE AU SEIN DE L'ARTICULATION TEMPORO-MANDIBULAIRE

Cinquante huit rats Sprague-Dawley ont recu une injection de thymidine tritiée à l'âge de quatre semaines et ont été sacrifiés au bout de périodes pariées allant jusqu'à 180 heures après l'injection, dans le but d'étudier les modifications du comportement des cellules des différents tissus de l'articulation temporo-mandibulaire. Les tissus ont été traités et soumis à l'analyse histologique et à l'analyse autoradiographique, ce qui a conduit aux conclusions suivantes:

1. Une différence certaine dans la duplication de l'ADN a été mise en évidence entre les populations cellulaires au sein de l'articulation temporo-mandibulaire. On a constaté dans la zone embryonnaire une élévation de l'indice de marquage, moins prononcé dans le fibrocartilage de la cavité glénoïde et la zone fibreuse du cartilage du condyle, et presque négligeable dans la partie biconcave du ménisque.

2. Les cellules de la zone embryonnaire présentaient un cycle d'une moyenne de 100 heures. Les phases S_1, G_2, M et G_1 avaient respectivement en moyenne une durée de 7, 1 ½, 1 ½, et 90 heures. Après division, 50 % des cellules environ demeuraient dans le pool progéniteur pour maintenir la population de réserve,

tandis que le reste des cellules se spécialisaient et devenaient des chondroblestes.

3. Aprés la différenciation, les chondrocytes marqués gardaient leur marquage, ce qui indiquerait une phase G_1 continuelle pendant leur migration dans le cartilage du condyle. Ces cellules sont finalement libérées dans la cavité osseuse après une vie de 5 à 7 jours.

4. La vitesse de renouvellement des cellules dans la zone articulaire du cartilage du condyle est relativement faible et semble être telle qu'elle assure le maintien de la surface articulaire et ne soit pas associée à la croissance au niveau du condyle. Les cellules marquées ont surtout leur origine dans les couches articulaires, à l'exception d'une contribution limitée de la zone embryonnaire.

5. Les indices de marquage du fbrocartilage de la cavité glénoïde et de la zone fibreuse du cartilage du condyle étaient une preuve du renouvellement des cellules au niveau des surfaces articulaires.

ZUSAMMENFASSUNG
ZELLULARE KINETIK INNERHALB DES KIEFERGELENKES

58 Spraque-Dewley Ratten im Alter von 4 Wochen wurde Tritium enthaltendes Thymidin injiziert. In verschiedenen Zeitstadien bis zu 180 Stunden nach der Injektion wurden sie obduziert, um Veränderungen im Verhalten der Zellen des Kiefergelenkes fastzustellen. Die Gewebe wurden für histologische und radioautographische Analysen präpariert. Es ergab sich folgendes:

1. Es wurde ein deutlicher Unterschied der DNS-Duplikation zwischen den zum Unterkiefergelenk gehörenden Zellgruppen konstatiert. In der embryonalen Schicht wurde ein grösserer Markierungsindex beobachtet, während sich der Gelenkfaserknorpel der Gelenkgrube und die Fibröse Schicht des Gelenkknorpels weniger beeinflusst zeigten. Im bikonkaven Teil des Discus articularis war der Index nahezu unerkennbar.
2. In den Zellen der embryonalen Schicht wurde ein Zellzyklus von durchschnittlich 100 Stunden festgestellt. Die S_1 G_2 M und G_1 Phasen dauerten durchschnittlich 7, 1 ½, 1 ½, bzw. 90 Stunden. Nach der Zellteilung blieben etwa 50 %

der Zellen in der Mutterzellgruppe als Reserven, während die anderen sich differenzieren und in Chondroblasten umwandeln.

3. Im Verlauf der Differenzierung behalten die markierten Chondrozyten ihre Markierung, was eine kontinuerliche G_1 Phase während der Migration im Knorpel des Kieferköpfchens vermuten lässt. Diese Zellen wandern schliesslich nach einer Lebenszeit von durchschnittlich 5 bis 7 Tagen in die Markhöhle ab.
4. Der Erneuerungsrhythmus der Zellen im Gelenkknorpel in der artikulierenden Fläche ist verhältnismässig langsam und geeignet, die Gelenkfläche unverändert zu erhalten. Für das Wachstum des Kieferköpfchens hat er keine Bedeutung. Die markierten Zellen stammten hauptsächlich von der artikulierenden Schicht her, ausgenommen eine geringe Zuwanderung aus der embryonalen Schicht.
5. Der Markierungsindex des bindegewebigen Gelenkfaserknorpels in der Gelenkgrube, wie auch der der fibrösen Schicht des Gelenkknorpels beweisen von einer Erneuerung in den artikulierenden Flächen.

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* abbreviations according to *Index Medicus*

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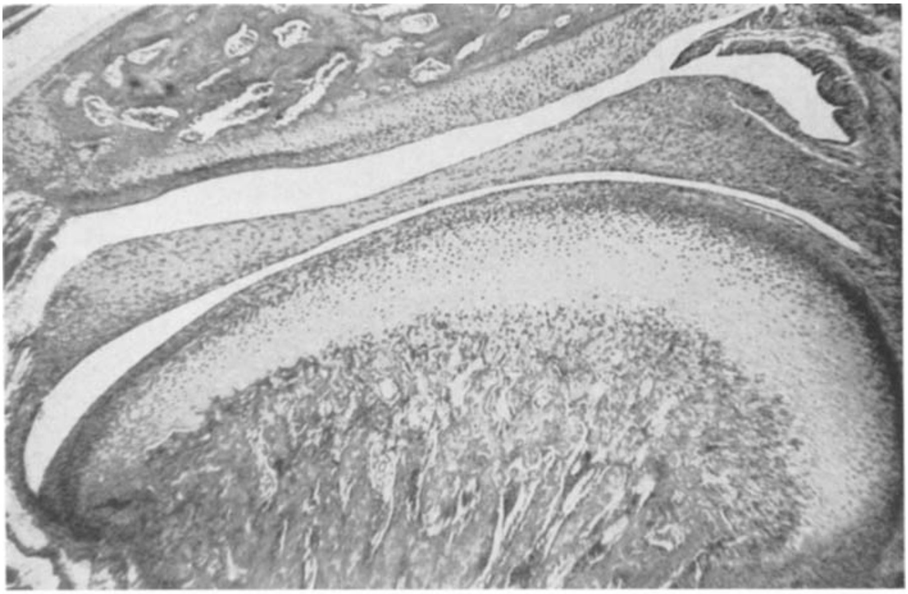
Fig. 1. Sagittal section of the squamosomandibular joint of a 4 week old rat. The condylar head is separated from the squamosal bone by the well-outlined articular disc. Synovial fringes are seen in the distal portion projecting into the superior synovial cavity. Mag. 48×.

Fig. 2. Photomicrograph illustrating the structural arrangement within (a) the articular cartilage of the squamosal bone, (b) the articular disc and (c) the condylar cartilage. Observe the cell density a few microns beneath the articular surface of the condylar head and the fossa. Mag. 240×.



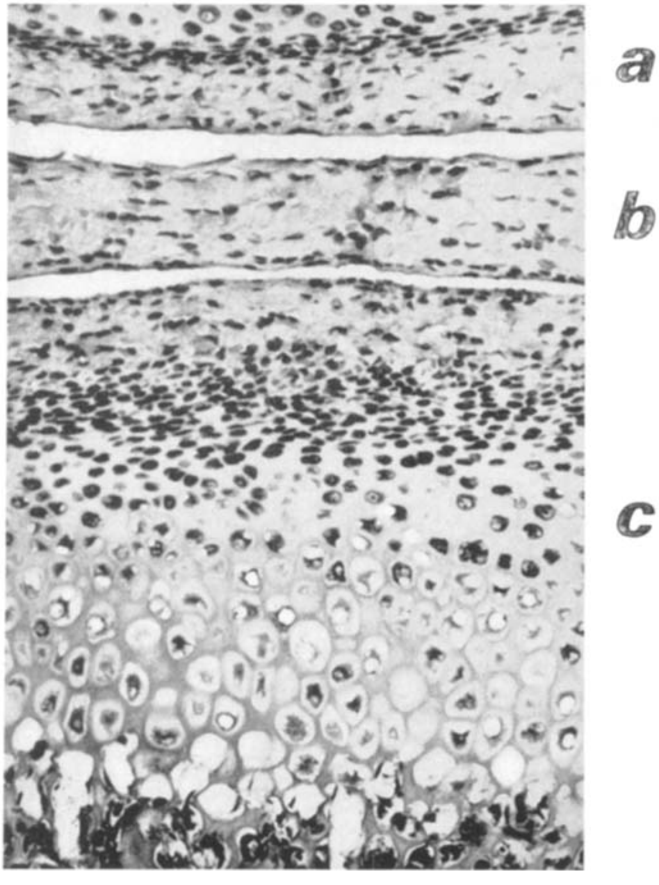

a
b
c

Fig. 3. Section through the condylar cartilage demonstrating the different zones. Mag. 540×.

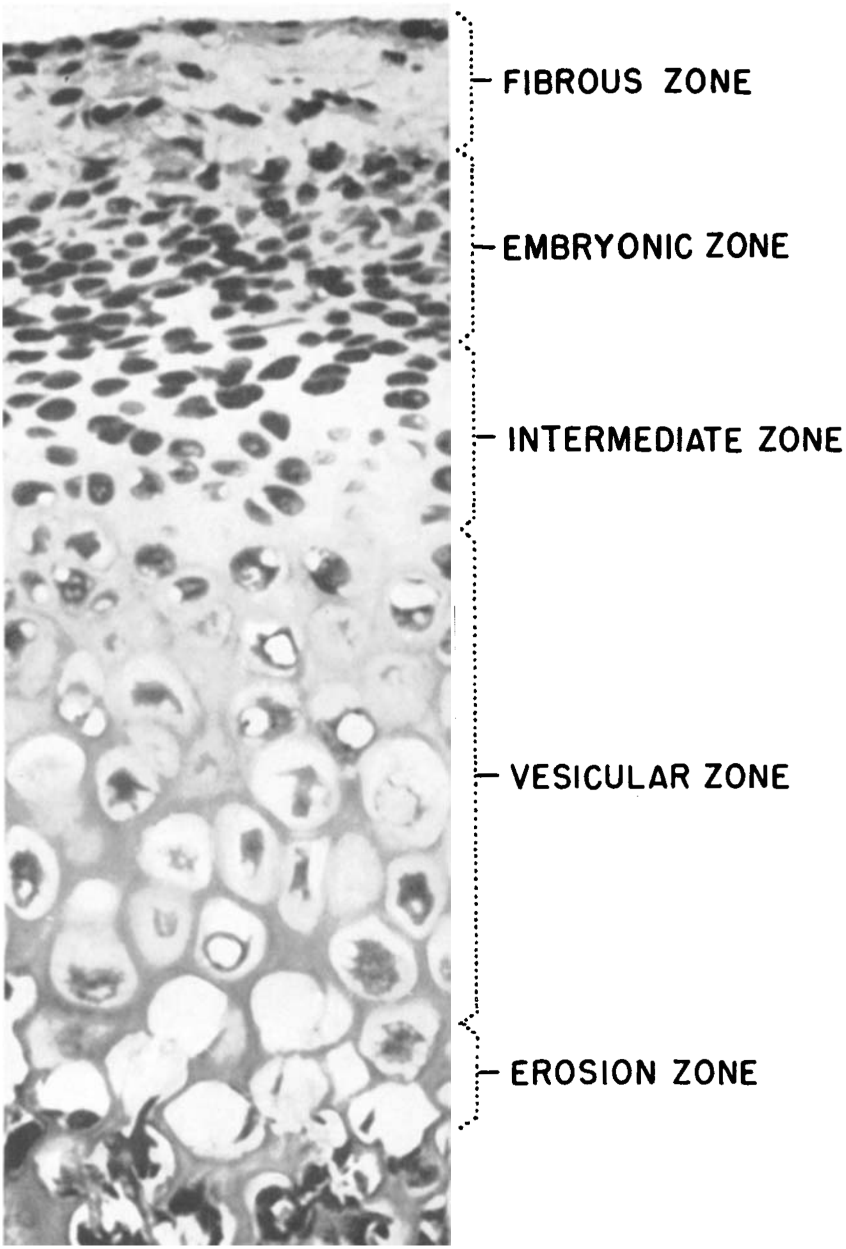

FIBROUS ZONE
EMBRYONIC ZONE
INTERMEDIATE ZONE
VESICULAR ZONE
EROSION ZONE

Fig. 4. Photomicrograph showing unlabeled mitotic figures within the embryonic zone of the condylar cartilage. Mag. 540×. Fig 4 a and b. Cells in metaphase with their chromosomes arranged at the equatorial plate, seen in a polar view (b) and in a side view (c). Mag. 750×. Fig. 4 d. Early telophase with partial cytoplasmic separation. Mag. 750×.

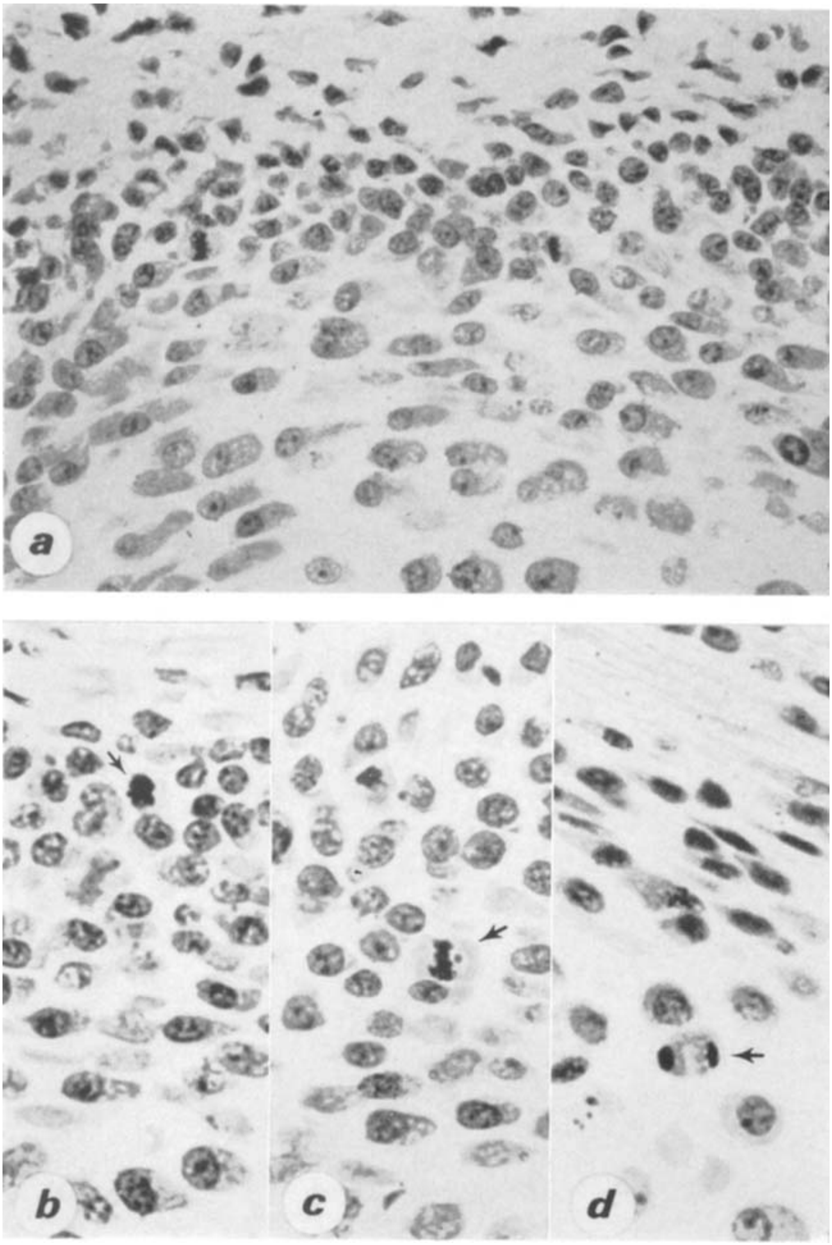

a
b
c
d

Fig. 5. Radioautograph demonstrating some labeled chondroprogenitor cells within the embryonic zone 1 hour after injection of tritiated thymidine. Observe the distinction of this layer from the overlying fibrous zone and the underlying vesicular zone. Mag. 540×.

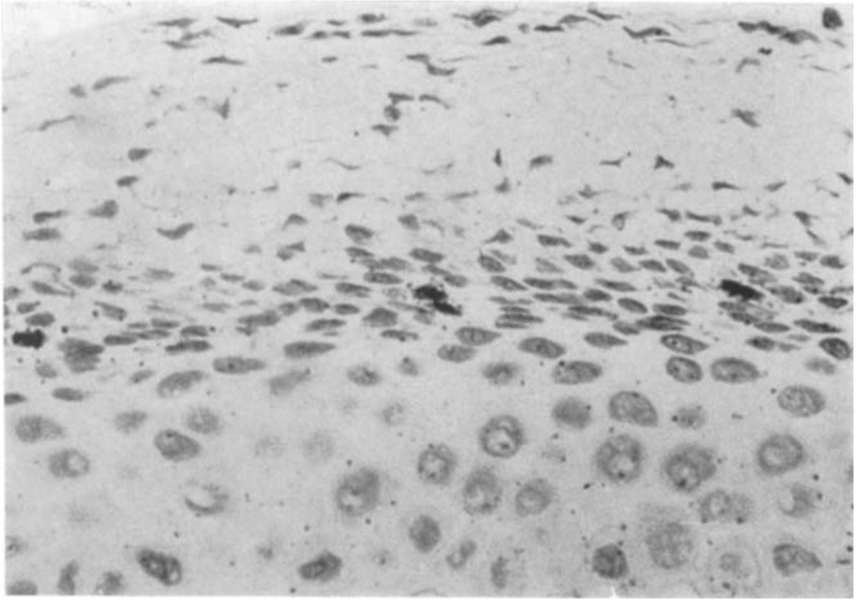


Fig. 6 a. Photomicrographs of the embryonic zone of the condylar cartilage demonstrating an unlabeled metaphase (Feulgen stain). Mag. 1200×. Fig. 6 b. Labeled cell in late metaphase or early anaphase (Hematoxylin) 2 hours after injection of tritiated thymidine. Mag. 1200×.

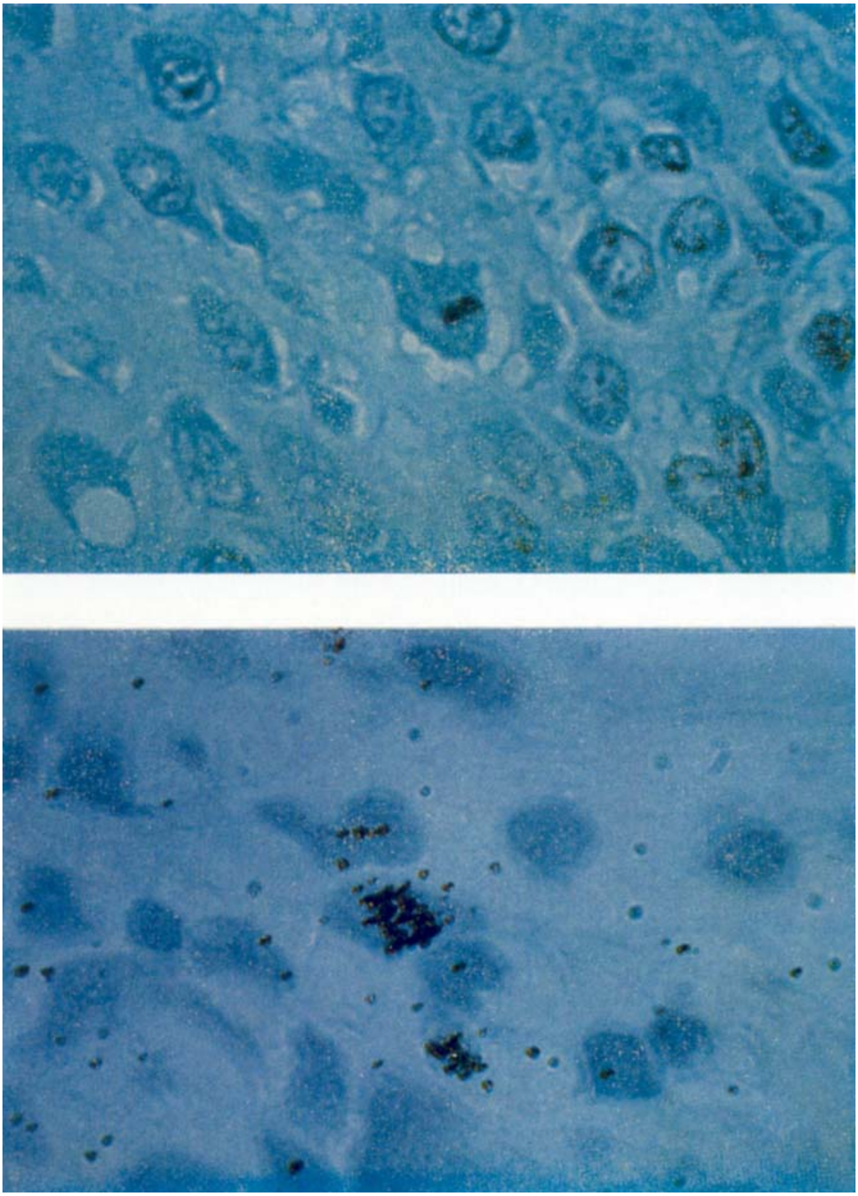
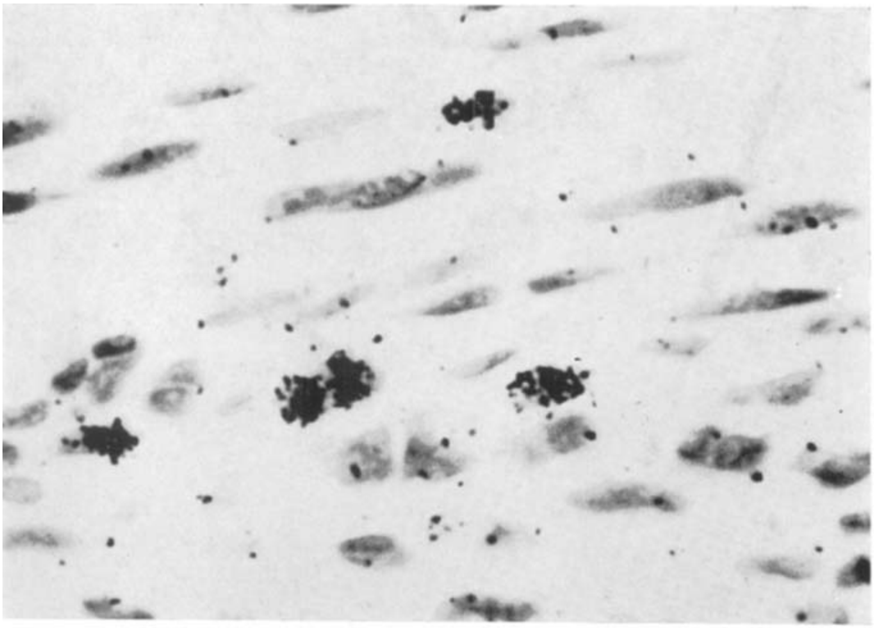
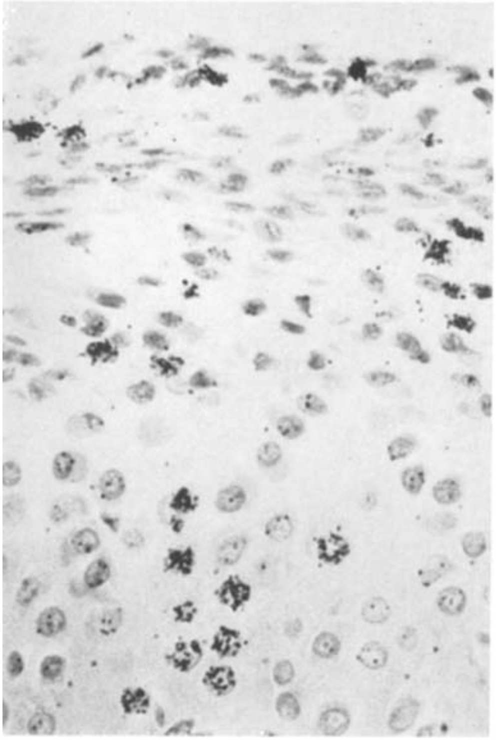


Fig. 7. Radioautograph of the embryonic zone showing a labeled telophase 3 hours after injection of tritiated thymidine. Mag. 1000×.

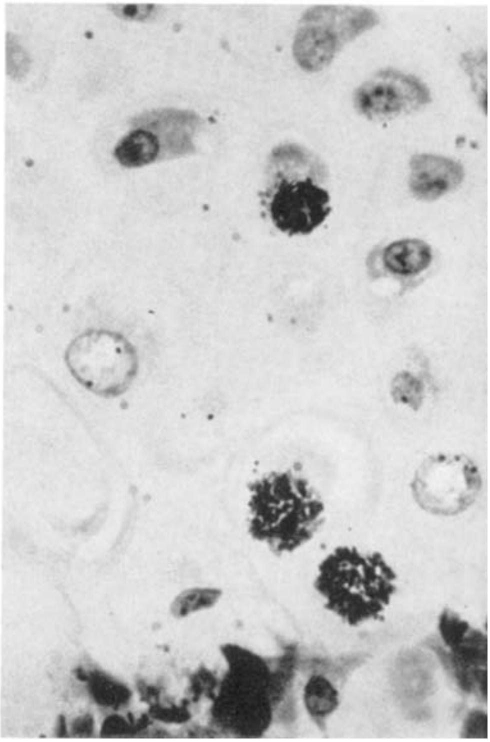
Fig. 8 a. Radioautograph of the condylar cartilage indicating the distribution of labeled cells 180 hours after injection of tritiated thymidine. Some of the cells have already reached the erosion zone and subsequently disintegrated. Mag. 350×.

Fig. 8 b. Radioautograph of the hypertrophic and erosion zones of the condylar cartilage demonstrating the high number of silvergrains localized over the nuclei of the chondrocytes suggesting that these cells remain undivided following their differentiation in the embryonic zone. Mag. 540×.





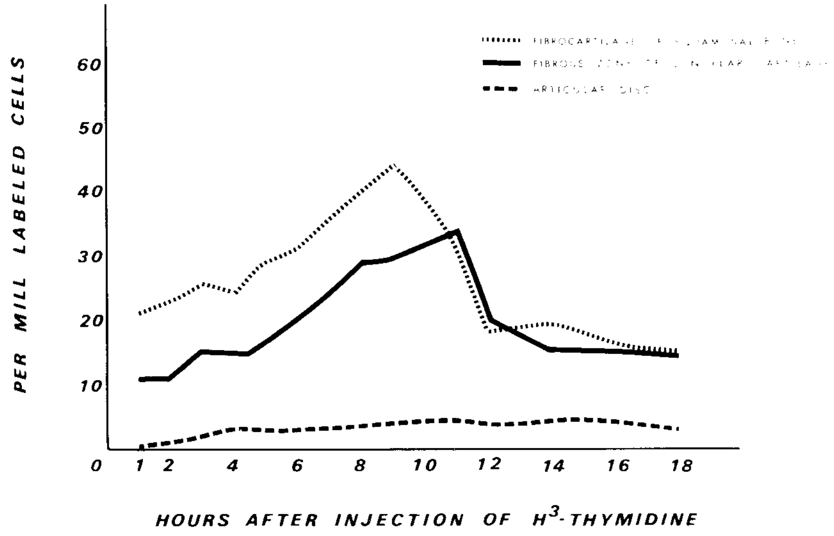
a



h

Chart 1. This graph is a comparison of the effect of time on the labeling index of the fibrocartilage of the squamosal bone, fibrous zone of the condylar cartilage, and the articular disc.

Chart 2. This graph illustrates the effect of time on the labeling index of the embryonic zone of the condylar cartilage.


FIBROCARTILAGE OF SQUAMOSAL BONE
FIBROUS ZONE OF CONDYLAR CARTILAGE
ARTICULAR DISC
PER MILL LABELED CELLS
60
50
40
30
20
10
0
1
2
4
6
8
10
12
14
16
18
HOURS AFTER INJECTION OF H^3-THYMIDINE

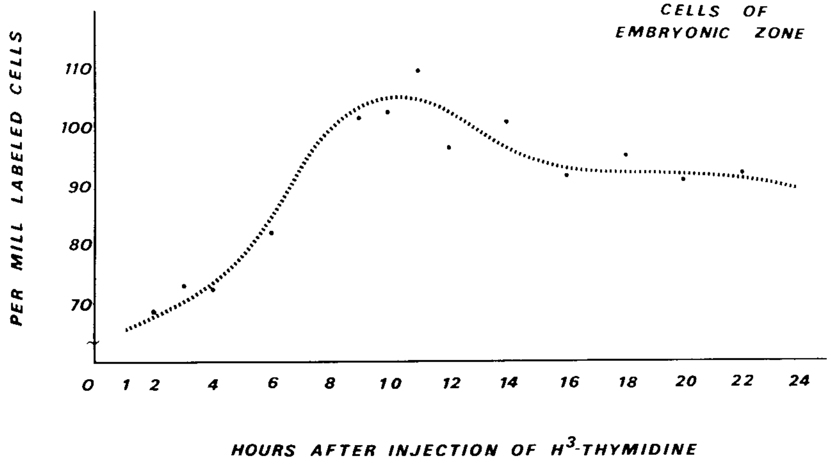
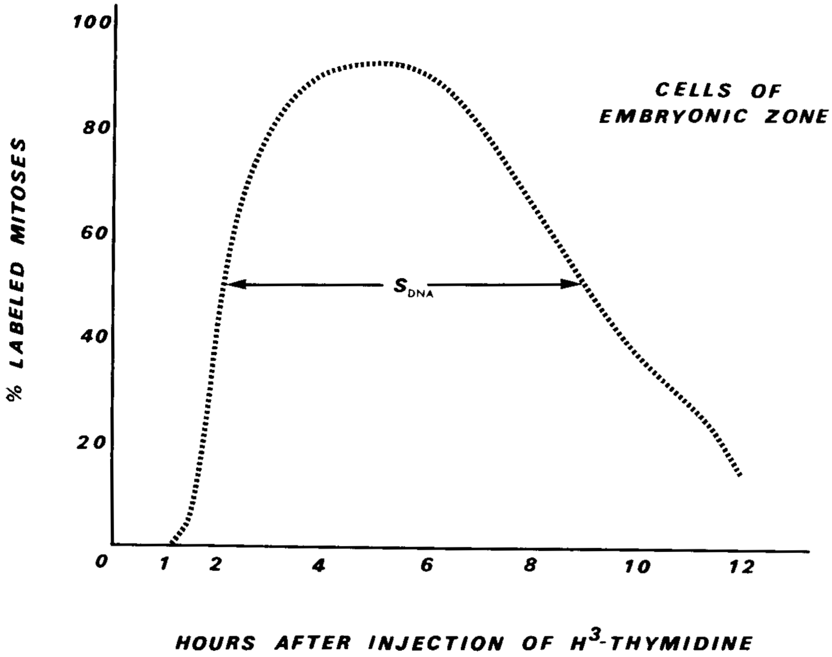

CELLS OF EMBRYONIC ZONE
PER MILL LABELED CELLS
110
100
90
80
70
0
1
2
4
6
8
10
12
14
16
18
20
22
24
HOURS AFTER INJECTION OF H^3-THYMIDINE

Chart 3. This diagram illustrates the distribution with time of labeled mitotic figures. The duration of the synthesis phase (S-DNA) was calculated by measuring the abscissal length between the 50 percent levels of ascending and descending limbs.

Chart 4. Diagram illustrating the events and average duration of the cell cycle within the embryonic zone of the condylar cartilage. Following DNA synthesis (S), the cells pass through G-2 and mitosis (M) to enter G-1. After division the daughter cells may remain in G-1 and eventually recommence the cell cycle or undergo modulation to become chondrocytes.


% LABELED MITOSES
100
80
60
40
20
0
1
2
4
6
8
10
12
CELLS OF EMBRYONIC ZONE
S_{DNA}
HOURS AFTER INJECTION OF H^3-THYMIDINE

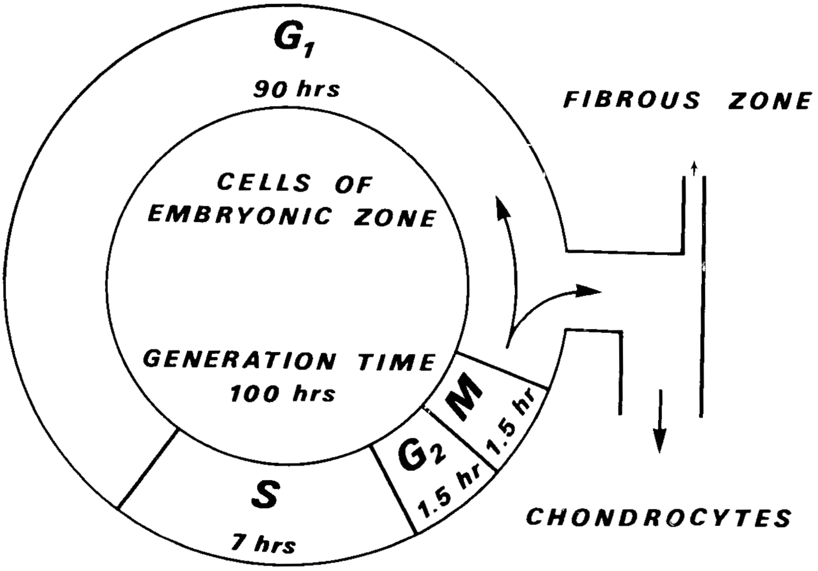

G_1
90 hrs
CELLS OF EMBRYONIC ZONE
GENERATION TIME
100 hrs
S
7 hrs
G_2
1.5 hr
M
1.5 hr
FIBROUS ZONE
CHONDROCYTES

Chart 5. Diagram illustrating the percental distribution of labeled cells in the condylar cartilage at 36 and up to 180 hours after administration of tritiated thymidine.

EFFECT OF TIME ON DISTRIBUTION OF LABELED CELLS