From:
The Department of Stomatognathic Physiology, University of Lund, the Department of Pathology I, University of Umeå, and the Department of Histology, Karolinska institutet, Stockholm, Sweden

COLLAGEN FORMATION AND GROWTH IN THE MANDIBULAR JOINT OF THE GUINEA PIG AS REVEALED BY AUTORADIOGRAPHY WITH 3H-PROLINE

by

TORSTEN ÖBERG
CARL-MARTIN FAJERS
ULF FRIBERG
STEFAN LOHMANDER

INTRODUCTION

Growth and maintenance of skeletal tissues are based upon two different processes, namely cell proliferation and matrix formation. Both these processes are difficult to follow with conventional histological techniques. However, they can be effectively visualized and studied by means of autoradiography. For example, cell proliferation and differentiation may be observed after labeling of nuclear DNA with ^{3}H-thymidine. The formation and deposition of matrices may be studied by using, e.g. a ^{3}H-labeled amino acid (which is incorporated into the structural protein, collagen) or ^{35}S-sulfate (which is incorporated into the sulfated glycosaminoglycan fraction of the amorphous ground substance). In such studies it is also possible to assess longitudinal and appositional growth by observing the apparent displacement of zones of labeled matrix, e.g. in relation to the erosion line and trabecular surfaces.

The approaches outlined have been utilized in a large number of skeletal studies, mostly dealing with long bones of rats and mice, and particularly with the endochondral process; for reviews see *Simmons* (1963), *Tonna et al.* (1963), *Tonna* (1965), and *Young* (1963). Only a few studies, however, have

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dealt with either the guinea pig or the mandibular joint. Cell proliferation in the mandibular joint was examined in the rat by *Blackwood* (1966) and *Folke & Stallard* (1966), and in the guinea pig by *Öberg et al.* (1967) using ^{3}H-thymidine. *Öberg* (1964) studied matrix formation with ^{35}S-sulfate in the mandibular joint of guinea pigs of different ages.

The present paper, which is concerned with collagen formation in the mandibular joint of young guinea pigs during normal growth, represents a direct continuation of the investigations by *Öberg* (1964) and *Öberg et al.* (1967). It was, therefore, possible to correlate the findings with both other autoradiographic observations and the results obtained with other methods of examination. Tritiated proline was used as a precursor.

MATERIAL AND METHODS

Fifteen young, growing guinea pigs were used. They were all males; aged 5 weeks when the isotope was administered. They were maintained under the conditions utilized by *Öberg* (1964).

Tritiated proline (TRA. 82, 0.1—0.2 C/mM, Radiochemical Centre, Amersham) was given intraperitoneally in a dose of 1 μC/g body weight. The animals were killed 10, 20 and 45 minutes, 1 ½ ,3, 6, 12 and 24 hours, and 2, 4, 6, 8, 17, 32 and 48 days after injection.

The mandibular joint preparations were fixed in 5 per cent formaldehyde buffered to pH 7,4 with sodium barbiturate and decalcified in 0.5 M diso-

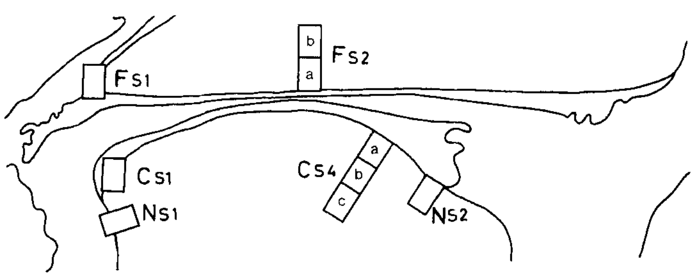

Fig. 1. Diagrammatic representation of a sagittal section through the mandibular joint, showing areas selected for high-power reproduction.
F s1 anterior border of fossa
F s2 central part of fossa
C s1 anterior border of condyle
C s4 posterior part of condyle
N s1 anterior border of neck
N s2 posterior border of neck
Modified from Öberg (1964). × 10.

dium ethylenediamine tetracetate (Na_2H_2-EDTA) at pH 8.0. After rinsing the preparations in 0.9 per cent sodium chloride, pH 7.4, they were embedded in paraffin. Sagittal sections were cut at 5 μm, mounted on glass slides, deparaffinized and coated with celloidin.

Ilford G5 emulsion was used for autoradiography; *Öberg*'s modification (1964) of the coating technique of *Messier and Leblond* (1957) was applied. The autoradiograms were exposed from 9 to 133 days. Before mounting, some of the slides were stained faintly with toluidine blue.

In order to show clearly the distribution of silver grains in unstained preparations, the latter were studied and photographed in both phase contrast (focus on section) and bright field (focus on grains).

On the basis of *Öberg*'s detailed observations on the structure of the joint, interest was mainly focused, when examining the autoradiograms, on the areas outlined in Fig. 1. The tissue layers in the mandibular joint were categorized according to *Öberg*. For the classification of bone cells *Young*'s (1962c) nomenclature and criteria were adopted, cf. *Öberg et al.* (1967).

Longitudinal growth was assessed quantitatively by measurements on autoradiograms of 8-day and 17-day specimens. With the aid of a measuring eyepiece, the distances were determined between the surface of the bone plate (fossa) or the erosion line (condyle) — as seen histologically — and the former location of these structures at the time of injection — as seen autoradiographically. Three different measurements were made on several sections from each joint preparation. Daily growth rates could then be calculated.

RESULTS

The autoradiographic observations are exemplified in Fig. 2 (overall view of the joint) and Figs. 3—7 (details).

The following three main events of matrix formation and growth may be recognized from the autoradiograms. First, the uptake of the isotope by the various connective tissue cells, and the subsequent secretion, to the matrices, of a pulse of labeled material, representing the bulk of the radioactivity. Second, growth as revealed by the apparent displacement of zones of labeled matrix due to the superimposition of nonlabeled matrix. Third, the ultimate elimination of labeled matrices.

In addition, there was also evidence of the skeletal cells utilizing some of the tritiated proline for the synthesis of intracellular proteins, e.g. in the persistence of cellular radioactivity for a few days after extrusion of the pulse of labeled matrix.

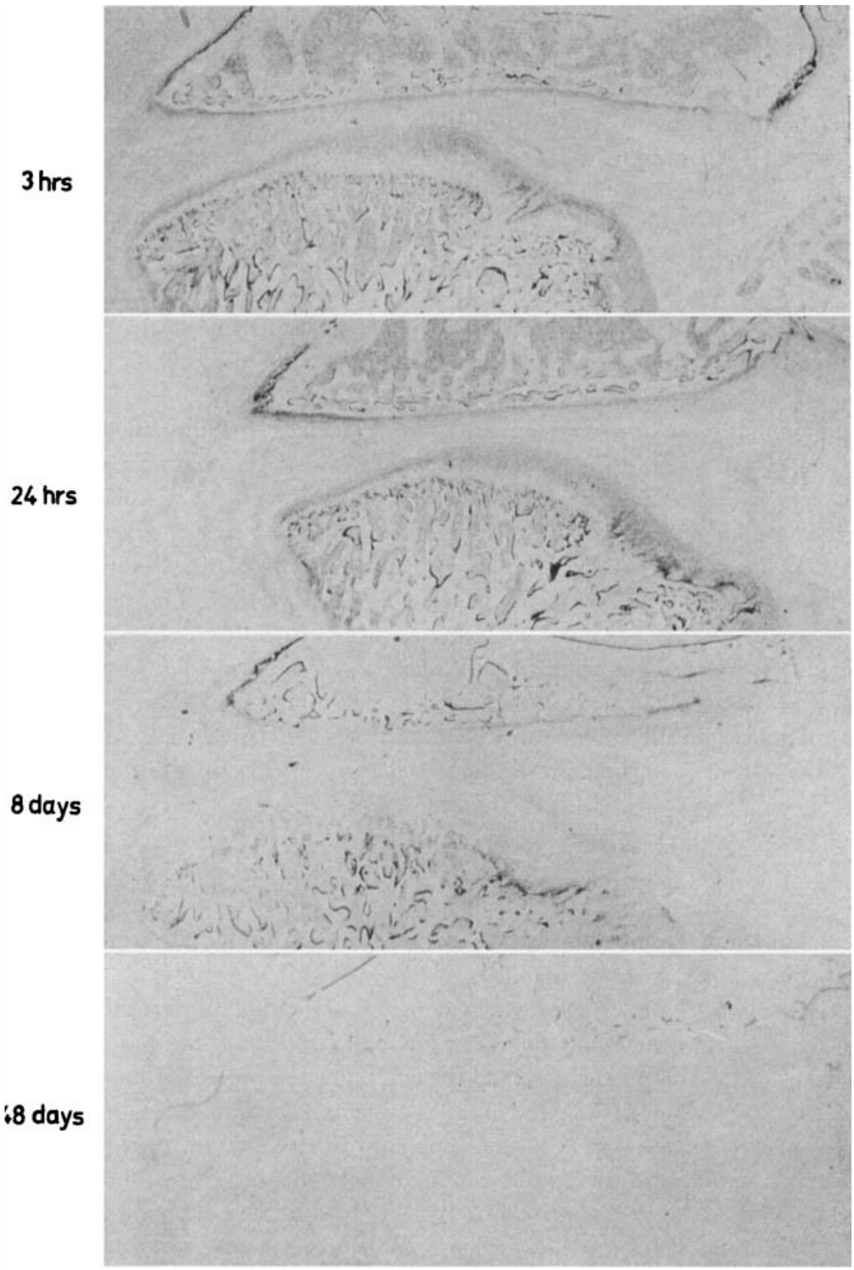

Fig. 2. Autoradiograms showing the overall distribution of ^{3}H-activity in the mandibular joint. The strongest uptake of ^{3}H-proline occurred in bone. Moderate incorporation in cartilage and bone marrow. The uptake and turnover of the isotope was more intense in the condyle, particularly the posterior part, than in the fossa. Exposure 133 days. × 12.

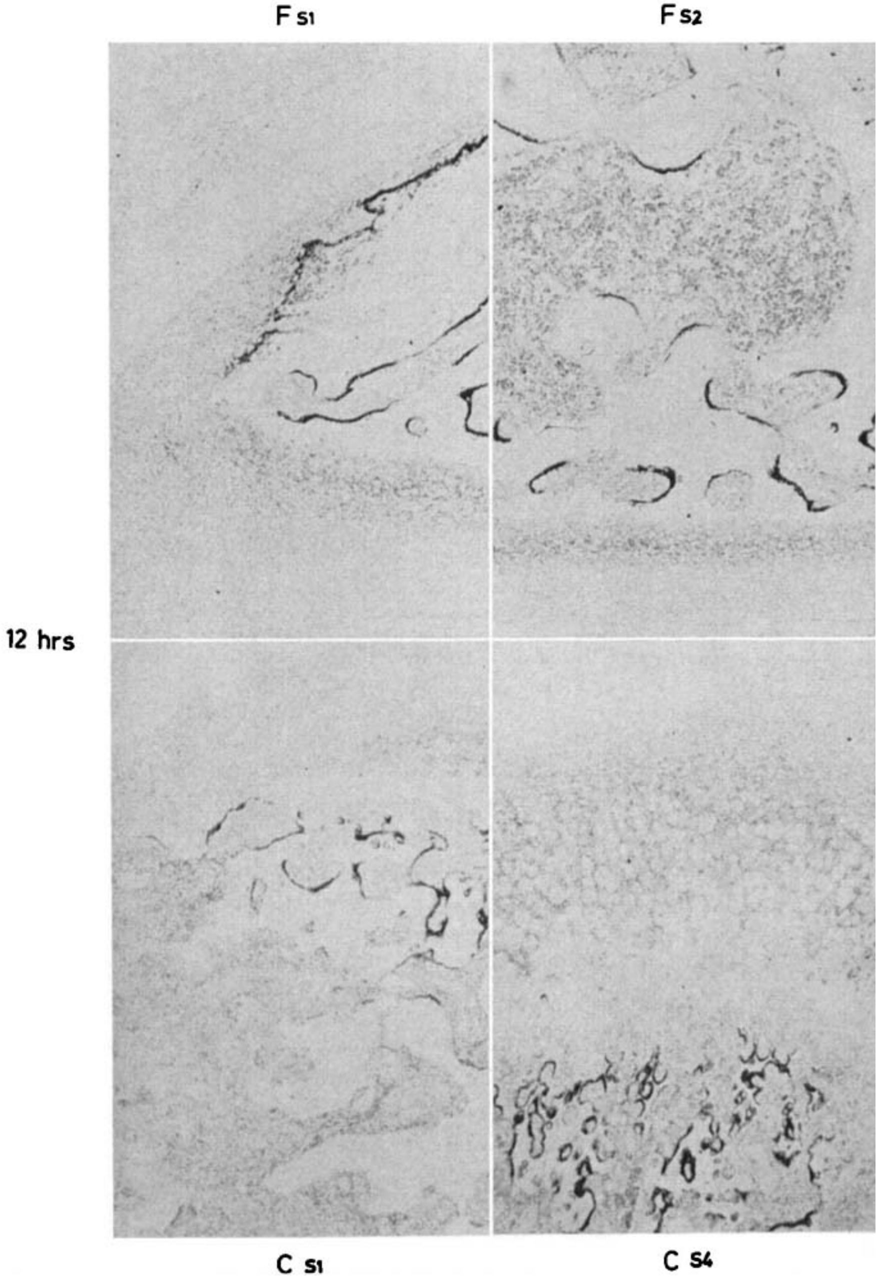

Fig. 3. Autoradiograms of fossa and condyle showing the initial tissue layer distribution of ^{3}H-activity. The highest radioactivity occurred in osteoblastic layers. Moderate ^{3}H-activity was observed in bone marrow and in the growth layers. Exposure 133 days. × 80.

N S1 N S2

6 hrs

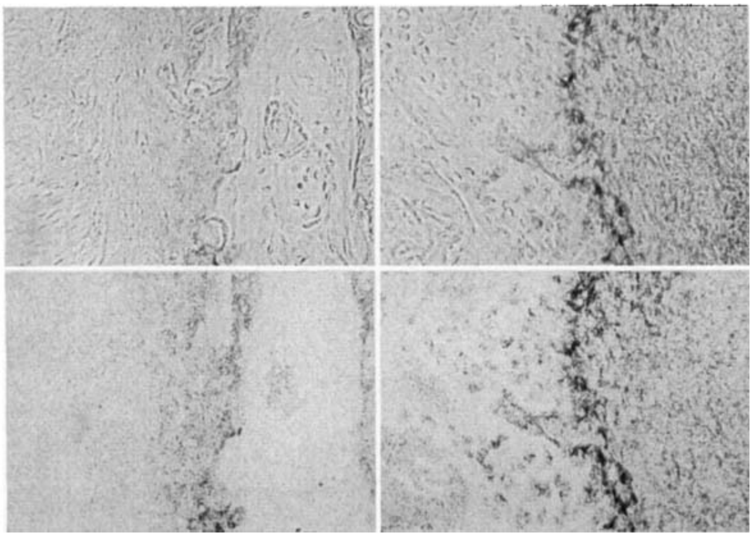

Fig. 4. Autoradiograms of the neck. The top frame of each pair in phase-contrast. The labeling with ^{3}H-proline was stronger at the posterior border, where osteoblastic activity predominates, than at the anterior border, where the bone is undergoing osteoclastic resorption. Note that osteocytes have incorporated ^{3}H-proline. Exposure 133 days. × 130.

Matrix formation. The initial uptake of ^{3}H-proline was most intense in osteoblasts (Figs. 2, 3). It was generally more marked, and involved a greater percentage of the cells, in the condyle than in the fossa. Particularly striking was the uptake of ^{3}H-proline by the tall osteoblasts of the superficial spongiosa in the posterior part of the condyle (Fig. 5). In this location, cellular ^{3}H-activity was distinctly observed already after 10 minutes and reached a maximum at 1 ½ to 3 hours. By this time the tritium activity was confined mainly to the apical part of the cytoplasm; some ^{3}H-activity could be observed also in the bone matrix immediatly adjacent to the cells. The secretory process was completed after 12 to 24 hours. In areas of less vigorous growth, however, e.g. in the deep condylar spongiosa, the transfer of labeled material did not appear to be complete until after 2 days.

Low to moderate uptake of ^{3}H-proline was observed in young osteocytes of newly formed bone in the fossa, the condyle, and the neck (Figs. 4—6). A slight uptake of the isotope occurred in osteoprogenitor cells and osteoclasts. There was no evidence of secretion of labeled material from these cells.

In general, the next highest uptake of ^{3}H-proline took place in hypertrophying chondrocytes, and was most pronounced in the growth areas, i.e.

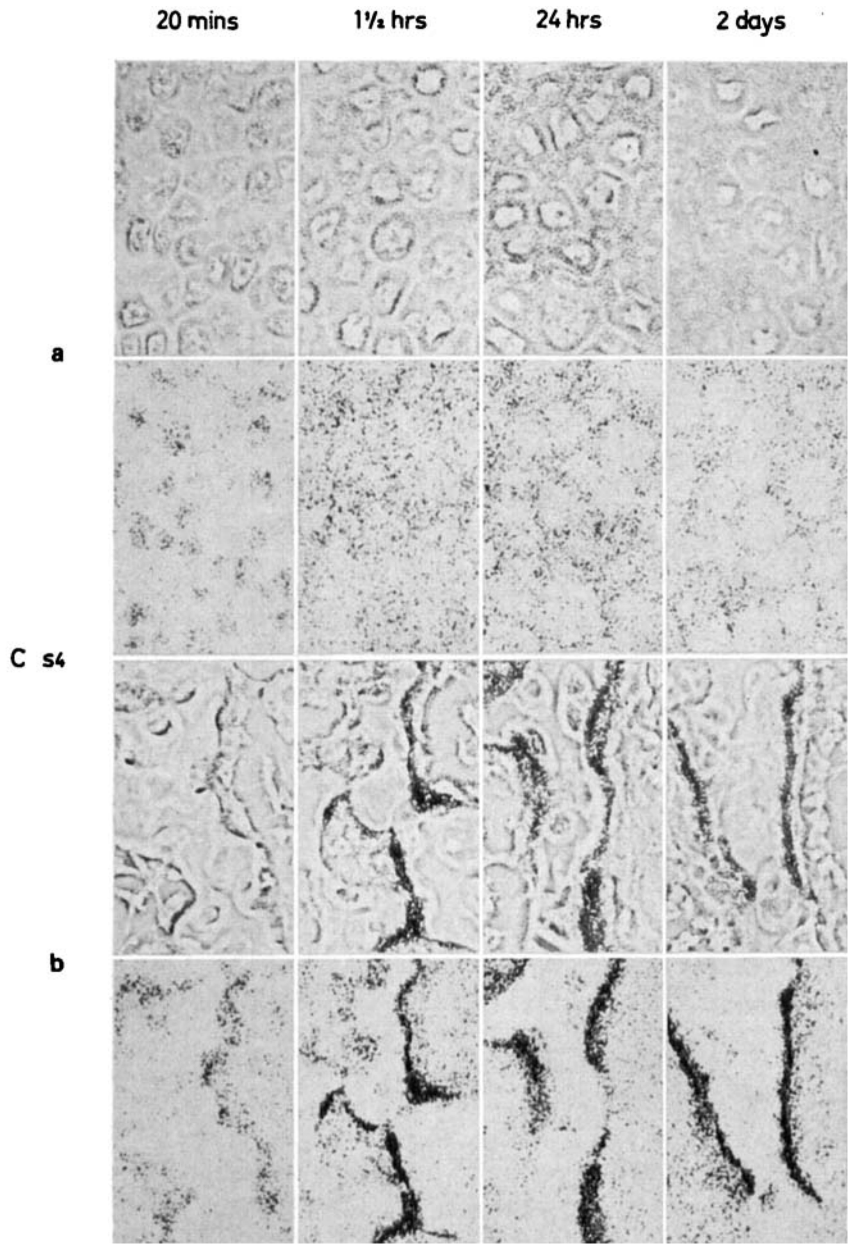

Fig. 5. Autoradiograms of posterior part of the condyle showing the hypertrophying cartilage (a) and the superficial part of the spongiosa (b). The top frame of each pair in phase-contrast. Incorporation and secretion of the isotope by chondrocytes and osteoblasts are clearly seen. Exposure 133 days (a) and 61 days (b). × 205.

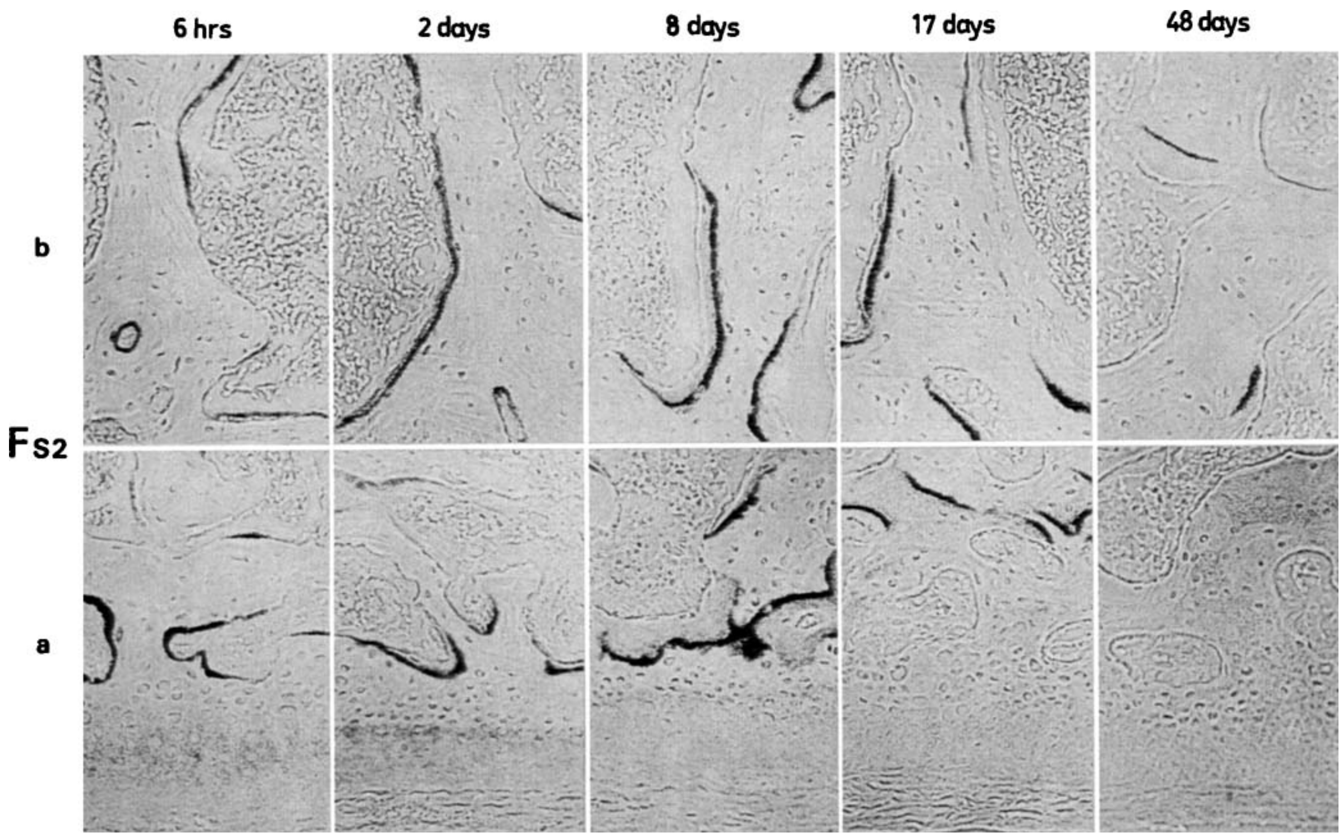

Fig. 6. Autoradiograms of the central part of the fossa, levels a & b. Phase contrast. The intensely radioactive bands, representing formation of lamellar bone, became located progressively further up in the spongiosa to be eliminated in the breakdown of old trabeculae (17—48 days); sequence a. In the deep spongiosa the rate of growth and remodeling was slower than closer to the articular surface; sequence b. Exposure 61 days. × 130.

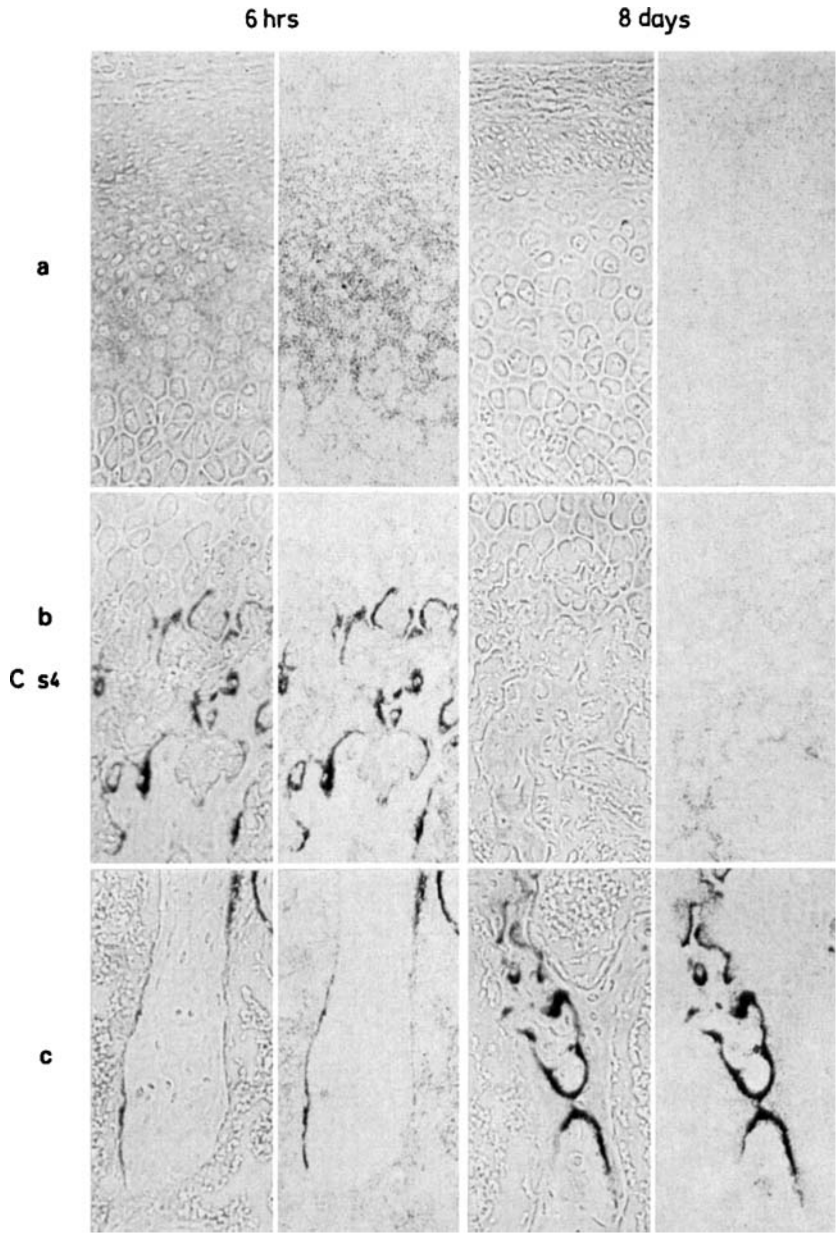

Fig. 7. Autoradiograms of the posterior part of the condyle, levels a—c. The left frame of each pair in phase contrast. The downward displacement of the radioactivity over an 8 day period corresponds to the combined thickness of the undifferentiated mesenchyme, transitional layer, and cartilage. Exposure 133 days. × 130.

anterior border of the fossa and the posterior part of the condyle (Figs. 2, 3). In the latter location, distinct ^{3}H-activity was observed within 20 minutes (Fig. 5). Maximum cellular radioactivity was reached between 45 minutes and 1 ½ hours. The transfer of the ^{3}H-activity to the matrix of the cartilage occurred even more rapidly than in the most active osteoblasts. Already after 45 minutes the appearance of ^{3}H-activity in the intercellular compartment was evident, and the secretion was essentially completed within 12 hours.

In calcifying cartilage, the chondrocytes of the superficial zone showed some uptake of ^{3}H-proline, whereas in the deep zone of fully mineralized cartilage, no incorporation of the isotope took place (Figs. 3, 7). It was not possible, by means of the autoradiograms, to establish any transfer of the ^{3}H-activity in the calcifying cartilage.

The uptake of ^{3}H-proline, by the cells of the undifferentiated mesenchyme, differed considerably in intensity between fossa and condyle (Figs. 2, 3). In the fossa, where the undifferentiated mesenchyme is associated with the formation of surface bone, the intensity of the radioactivity approached, and in the anterior part even surpassed, that of the condylar cartilage. In the condyle, where the undifferentiated mesenchyme gives rise to transitional-layer cells and chondrocytes, the ^{3}H-activity was low. Since the cells are small and closely packed in the undifferentiated mesenchyme, it was difficult to follow, in detail, the transfer process in this layer. However, ^{3}H-activity was first observed in the matrix after 3 hours, and in 1—2 days the activity was predominantly extracellular.

The incorporation of tritiated proline by the cells of the transitional layer was intermediate between that of the undifferentiated-mesenchyme cells and that of the hypertrophying chondrocytes (Fig. 3).

The initial uptake of ^{3}H-proline was low in the connective tissue linings, the disc, and the capsule (Figs. 2, 3). In these layers it was also difficult to follow the transfer process, with the exception of the central part of the disc where chondroid cells occur.

Growth. The deposition of a pulse of labeled matrix in a given tissue layer is followed by the secretion of non-labeled material. Hence, new tissue layers formed in the continuous process of skeletal growth will not be radioactive. In the autoradiograms this is visualized as a displacement of bands of radioactivity away from the articular surfaces. Thus, in the fossa a band of ^{3}H-activity was observed to pass from the undifferentiated mesenchyme, through the compact bone plate, to be broken up in the spongiosa (Fig. 6); another band passed through the mineralized cartilage at the anterior border. Concomitantly, the strongly labeled bands of trabecular bone moved progressively further into the spongiosa. From the displacement of these bands during the first 17 days after administration of tritiated proline, the daily growth rate was calculated to be about 5 μm in the central part of the fossa.

In the condyle two radioactive bands, separated by an essentially non-active band, moved *en bloc* down into the spongiosa (Fig. 7). These bands corresponded to hypertrophying cartilage (plus undifferentiated mesenchyme and transitional layer), calcifying cartilage (essentially non-active), and superficial spongiosa respectively, at the time when the ^{3}H-proline was administered. From the displacement of these bands during the first 17 days, a daily growth rate of about 45 μm was calculated for the posterior part of the condyle.

Similarly, the deposition of non-labeled bone matrix on labeled matrix in trabecular bone may readily be followed in the autoradiograms (Fig. 6). In the fossa, trabecular apposition was about 4—5 μm per day in the superficial spongiosa, and about 2 μm in the deep spongiosa. In the posterior part of the condyle the daily apposition was about 8—10 μm in the superficial spongiosa, and about 4—5 μm in the deep spongiosa (Fig. 7).

Elimination of labeled matrix. Labeled collagen, deposited by the osteoblasts of the spongiosa, is quickly entrapped by the calcification of the bone matrix (Figs. 6, 7). In the growth layers the radioactivity of the matrices is similarly »frozen in place» already within 1—4 days by the rapidly progressing differentiation and mineralization of cartilage and bone. No loss of label was observed before calcification. The elimination of labeled matrices took place, therefore, in the breakdown of trabeculae and in the erosion of calcified cartilage (Figs. 2, 6, 7). Practically all of the ^{3}H-activity had disappeared from the condyle after 32 days. In the fossa, however, considerable amounts of labeled bone matrix were still present, except at the anterior border, after 48 days (Fig. 2).

In the connective tissue linings, the disc, and the capsule the radioactivity was eliminated at an exceedingly slow pace, so that after 48 days the ^{3}H-label of these layers was second only to that of bone (Fig. 2).

Uptake by non-skeletal cells. In the bone marrow, uptake of ^{3}H-proline, indicating incorporation of the isotope into intra-cellular proteins, occurred in the myeloid cells in general, including the megakaryocytes (Figs. 2, 3, 6). The uptake was noticeable after 20—45 minutes and increased until 12 hours, by which time it equalled that of cartilage. The radioactivity thereafter decreased rapidly; only weak activity still remained after 6—8 days.

A similar, moderate, and transitory uptake of the isotope was also observed in the secretory cells of the parotid gland, a retro-articular portion of which was included in some of the joint preparations.

DISCUSSION

There is no specific precursor that may be used to label collagen in autoradiographic work. However, previous studies on collagen formation in mesenchymal tissues have shown that either glycine (*Carneiro & Leblond*, 1959; *Young* 1962 a, b; *Tonna et al.* 1963) or labeled proline (*Ross & Benditt*, 1962; *Stallard*, 1963, 1964; *Crumley*, 1964) can be used as tracers for this purpose. From a strictly quantitative aspect, glycine is preferable, since it is the predominant amino acid of collagen, accounting for one-third of the total amino acid residues (*Piez & Likins*, 1960). Glycine, however, is known to undergo numerous metabolic transformations (*Meister*, 1965). Therefore, ^{3}H-proline was chosen for the present study, in order to minimize the possibility of inadvertent labeling of substances other than proteins. Biochemical work performed *in vivo* (*Stetten & Schoenheimer*, 1944; *Stetten*, 1949; *Hausmann & Neumann*, 1961) and *in vitro* (*Smith & Fitton-Jackson*, 1957; *Lowther, Green & Chapman*, 1961; *Peterkofsky & Udenfriend*, 1961) has demonstrated that both collagen-proline and collagen-hydroxyproline are derived from free proline, whereas free hydroxyproline is not utilized for the synthesis of collagen. Since proline and hydroxyproline form nearly one-fourth of the total amino acid residues of collagen, it follows that proline may be employed as a precursor for autoradiographic work without affecting sensitivity to any appreciable extent.

Previous autoradiographic studies have indicated that skeletal cells use large quantities of amino acids for the formation of matrix proteins, but relatively small amounts for the synthesis of intracellular proteins (*Tonna*, 1965). The present results confirm this. By far the greater part of the ^{3}H-activity taken up, by, e.g. osteoblasts and chondrocytes, was transferred rapidly to the matrices. In the non-secretory osteoprogenitor cells and osteoclasts the uptake of the isotope was low. This low degree of utilization of ^{3}H-proline for the formation of intracellular protein by the various skeletal cells contrasts markedly with that shown by the myeloid cells of the bone marrow.

It is thus evident that ^{3}H-proline may be used as an effective indicator of the formation and deposition of matrical protein. More specifically, it seems justifiable to assume that the protein thus visualized as being extruded into the matrices was, essentially, collagen, since by far the greatest portion of proline/hydroxyproline in skeletal matrices occurs in this protein. Elastin, which also contains substantial amounts of these amino acids (*Partridge* 1962), is not present in significant amounts in the tissues examined here. The protein component of the protein-polysaccharide complex (proteoglycans) of the ground substance is quantitatively less important than collagen, particularly in bone. Moreover, it has a low content of proline (*Gross et al.* 1960; *Campo & Dziewiatkowski,* 1962). Regarding bone, the findings of *Carneiro and Leblond* (1965) confirm that ^{3}H-proline and ^{3}H-glycine are transferred to the matrix, essentially as part of the collagen. Collagenase treatment of sections was used as specificity control by these authors.

With regard to the specificity of the matrix label in cartilage and bone, it is also of interest that the intensity of the label, as noted in the present autoradiograms, parallels the dry mass of the matrices, as demonstrated previously by microradiography (*Öberg,* 1964). Thus, in order of increasing ^{3}H-activity and dry mass we have: cartilage, surface bone, and lamellar bone. Furthermore, with ^{3}H-proline the strongest label occurred in osteoblasts and bone matrix, whereas with ^{35}S-sulfate the most intense radioactivity was observed in the chondrocytes and cartilaginous matrix (*Öberg,* 1964). This is in accordance with the different chemical composition of the matrices of bone and cartilage.

In general, the present findings concerning the utilization of ^{3}H-proline were in close agreement with the results obtained with ^{35}S-sulfate by *Öberg* (1964). First, with both precursors, a direct relationship was observed between cellular uptake and skeletal cell specialization. The strongest uptake occurred in hypertrophying chondrocytes and osteoblasts, e.g. cells directly engaged in matrix formation. On the other hand, there was a weak uptake in undifferentiated-mesenchyme cells and osteoprogenitor cells, which form the two major proliferative compartments in the mandibular joint (*Öberg et al.,* 1967).

Second, the time sequence for the cellular uptake of tritiated proline and deposition of labeled collagen paralleled that previously established for the synthesis and secretion of sulfated glycosaminoglycans of the ground substance. This temporal correspondence might be interpreted as an indication of a close relationship between the two synthetic pathways. Experimental evidence to the contrary has, however, been presented by *Bhatnagar & Prockop* (1966). They showed that, *in vitro,* either synthetic process can be blocked without affecting the other.

Third, both precursors showed the same regional variations in the intensity of matrix label within the joint. These variations are directly related to growth. Thus, the matrices were most intensely labeled in the growth centers at the anterior border of the fossa and in the posterior part of the condyle. In general, the matrices were more strongly labeled in the condyle than in the fossa.

Fourth, the values obtained with ^{3}H-proline for daily longitudinal growth and trabecular apposition were in complete accordance with those reported by *Öberg* (1964).

Summing up the results of the morphologic and autoradiographic observations in the present series of investigations the following interpretations of the growth process in the mandibular joint may be suggested. *Cell renewal* proceeds from two major proliferative compartments: the undifferentiated-mesenchyme cells and the osteoprogenitor cells. These cells actively incorporate ^{3}H-thymidine during DNA-synthesis prior to mitosis. The amounts of matrix produced by these cells are limited, however, as indicated by their low utilization of ^{35}S-sulfate and ^{3}H-proline, and by the low dry mass content of the intercellular substance. *Matrix formation* is also effected mainly by two cell populations, namely hypertrophying chondrocytes and osteoblasts. The productive nature of these cells is reflected in their pronounced uptake and transfer of both matrix precursors. Both cell types also are surrounded by matrices with a marked X-ray absorption. On the other hand, these cells do not take part in the proliferative process; they do not incorporate ^{3}H-thymidine.

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SUMMARY

Collagen formation and growth in the mandibular joint was studied by autoradiography with ^{3}H-proline in 5-week-old guinea pigs. The isotope was administered, in a dose of 1 μC/g, to 15 animals, killed at intervals varying from 10 minutes to 48 days following injection.

The initial uptake of ^{3}H-proline was most intense in osteoblasts, particularly in the posterior part of the condyle. The next highest uptake of tritiated proline occurred in hypertrophying chondrocytes. In both cell types distinct ^{3}H-activity was observed already after 10—20 minutes, with a maximum at about 1 ½ hours. Secretion of labeled collagen was completed after 12—24 hours.

Growth was assessed quantitatively by measurements on autoradiograms from 8-day and 17-day specimens. The daily growth rate was calculated to about 5 μm in the central part of the fossa and to about 45 μm in the posterior part of the condyle. Trabecular apposition amounted to 2—5 μm per day in the fossa and 4—10 μm in the condyle.

Elimination of labeled matrices took place mainly in the breakdown of trabeculae and in the erosion of calcified cartilage. Practically all of the ^{3}H-activity had disappeared from the condyle after 32 days. In the fossa, how-

ever, considerable amounts of labeled bone matrix were still present after 48 days.

The present findings on the utilization of ^{3}H-proline were, in general, in close agreement with the results obtained previously with ^{35}S-sulfate, and were also correlated to previous microradiographic observations.

Characteristic differences between the proliferative and productive cell types of the mandibular joint, as shown by the present series of investigations, are discussed.

RÉSUMÉ

FORMATION ET CROISSANCE DU COLLAGÈNE DANS L'ARTICULATION MANDIBULO-TEMPORALE DU COBAYE, D'APRÈS L'ÉTUDE AUTORADIOGRAPHIQUE AVEC LA PROLINE-^{3}H

La formation et la croissance du collagène dans l'articulation mandibulo-temporale ont été étudiées par autoradiographie avec la Proline-^{3}H chez des cobayes âgés de 5 semaines. L'isotope a été administré à la dose de 1 μc/g à 15 animaux qui ont été sacrifiés à des intervalles variant de 10 minutes à 48 jours après l'injection.

L'absorption initiale la plus intense de proline-^{3}H se faisait dans les ostéoblastes, particulièrement à la partie postérieure du condyle. Le degré le plus élevé d'absorption de la proline tritiée est ensuite constaté dans les chondrocytes s'hypertrophiant. Dans ces deux types de cellules, on pouvait déjà nettement observer l'activité ^{3}H au bout de 20 minutes, avec un maximum au bout d'environ 1 heure 1/2. La sécrétion du collagène marqué était terminée au bout de 12—24 heures.

L'évaluation quantitative de la croissance a été faite par des mensurations sur des autoradiographies de spécimens de 8 jours et de 17 jours. La croissance journalière calculée était d'environ 5 μm à la partie centrale de la cavité glénoïde et d'environ 45 μm à partie postérieure du condyle. L'apposition trabéculaire était de 2—3 μm par jour dans la cavité glénoïde et de 4—10 μm dans le condyle.

L'élimination des matrices marquées se faisait principalement pendant la destruction des trabécules et pendant l'érosion du cartilage calcifié. Toute l'activité ^{3}H avait pratiquement disparu du condyle au bout de 32 jours. Mais dans la cavité glénoïde, des quantités notables de matrice osseuse marquée étaient encore présentes au bout de 48 jours.

Les résultats obtenus ici sur l'utilisation de la proline-^{3}H étaient dans l'ensemble tout-à-fait conformes aux résultats obtenus antérieurement en utilisant

un sulfate-^{35}S, ainsi qu'aux observations microradiographiques faites antérieurement.

Les différences caractéristiques mises en évidence par la présente étude entre les cellules de type prolifératif et de type productif font l'objet d'une discussion.

ZUSAMMENFASSUNG

AUTORADIOGRAPHISCHE STUDIEN MIT ^{3}H-PROLIN ÜBER DIE BILDUNG VON KOLLAGEN UND DEN ZUWACHS IM KIEFERGELENK VON MEERSCHWEINCHEN

Die Bildung von Kollagen und der Zuwachs im Kiefergelenk von fünf Wochen alten Meerschweinchen wurden autoradiographisch mit ^{3}H-Prolin studiert. Fünfzehn Tiere erhielten eine Dosis von 1 μC/g und wurden zwischen 10 Minuten und 48 Tagen nach der Injektion getötet.

Die unmittelbare Aufnahme von ^{3}H-Prolin war am stärksten in den Osteoblasten, besonders im hinteren Teil des Kondylus. Die zweitstärkste Aufnahme wurde in den Chondrozyten der Hypertrophiezone gefunden. In beiden Zellgruppen konnte eine deutliche Aktivität schon nach 10 bis 20 Minuten festgestellt werden, die nach 1 ½ Stunden ein Maximum erreichte. Die Ausscheidung von radioaktivem Kollagen war nach 12 bis 24 Stunden abgeschlossen.

Der Zuwachs wurde quantitativ mit Hilfe von Autoradiogrammen von Tieren, die acht und siebzehn Tage nach der Injektion getötet worden waren, gemessen. Die tägliche Zuwachsrate betrug ungefähr 5 μm im zentralen Teil der Fossa und ungefähr 45 μm im hinteren Teil des Kondylus. Die trabekuläre Apposition erreichte Werte von 2 bis 5 μm in der Fossa und 4 bis 10 μm im Kondylus.

Die Elimination von radioaktiver Matrix fand hauptsächlich beim Abbau der Trabekeln und bei der Erosion des verkalkten Knorpels statt. Die ^{3}H-Aktivität im Kondylus war nach 32 Tagen fast vollständig verschwunden. In der Fossa konnten jedoch auch nach 48 Tagen beträchtliche Mengen von radioaktiver Knochenmatrix gefunden werden.

Die vorliegenden Untersuchungsresultate über den Umsatz von ^{3}H-Prolin stimmen gut mit den Ergebnissen von früheren Untersuchungen überein, bei denen ^{35}S-Sulfat verwendet wurde. Ferner finden wir eine gute Übereinstimmung mit mikroradiographischen Ergebnissen.

Die Unterschiede zwischen proliferativen und produktiven Zelltypen des Kiefergelenks, wie sie in einer Serie von Arbeiten nachgewiesen worden sind, werden diskutiert.

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Addresses:

Torsten Öberg,
Department of Stomatognathic Physiology,
University of Lund,
School of Dentistry,
Malmö S,
Sweden

Carl-Martin Fajers,
Department of Pathology I
University of Umeå,
Umeå,
Sweden

Ulf Friberg, Stefan Lohmander,
Department of Histology,
Karolinska institutet,
Stockholm,
Sweden