From: The Department of Stomatognathic Physiology, School of Dentistry and Departments of Pathology I and II, University of Umeå, Umeå, Sweden.

MICRORADIOGRAPHIC STUDY OF THE MANDIBULAR DISK IN THE GUINEA PIG

by

T. Öberg

G. E. Carlsson

T. Ekberg

O. Hassler

INTRODUCTION

Temporomandibular joint disks obtained at operation and autopsy have been reported to display various kinds of alterations, including perforation and fraying, calcification and hyaline and fibrinoid degeneration. The reported frequencies of such changes vary widely. As to calcification, in an autopsy series of 42 joints from persons ranging in age from 3 months to 81 years *Bauer* (1941) found one disk in particular with extensive calcification of its dystrophic tissue. In an operation series of 58 disks, 38 of which were subjected to histologic examination, *Boman* (1947) found one with degenerative alterations and calcareous deposits. These studies thus suggest that calcification in the disk is uncommon. In persons of advanced age, however, *Macalister* (1954) found degenerative alterations and calcification to occur fairly often in a histologic examination of 69 joints from deceased persons aged 16—86 years.

More recently it has been discussed how changes, including degenerative calcification, in the actual articular tissues might give

rise to pain and other symptoms of dysfunction in the temporomandibular joint (*Toller,* 1961, *Moffett,* 1962, *Blackwood,* 1963, *Davidson,* 1964).

The present study was carried out with the purpose of assessing the value of the soft X-ray technique for visualizing calcification in the mandibular disk, and of finding the prevalence of such changes in the disk of the guinea pig over a range of age.

MATERIAL

The study was performed on 36 mandibular disks from 18 guinea pigs (9 male, 9 female) in 5 age groups (Table I). The animals were of the same mixed stock and were obtained from a commercial breeder (J. Sahlin, Kvarnby, Sweden). The guinea pigs were apparently in sound health and displayed no irregularities in the dentition.

Table I

Distribution of the material

Age group	1 mo	6 mo	12 mo	18 mo	4 yr	Total
No. of guinea pigs	2	4	4	3	5	18

METHOD

The guinea pigs were sacrificed by inhalation of ether. The joints were dissected as reported by *Öberg* (1964). The disks were removed and placed together in a polythene bag after noting the orientation.

Soft X-ray examination. — Contact microradiography was performed immediately the specimens had been prepared. A Philips fine focus tube 25633/62 was used, with a copper anode and a nickle filter; the constants were 7.5 kV and 50 mA. The exposure was varied from 30 to 60 minutes, depending on the thickness of the specimen. The film—focus distance was 23 cm. Gevaert Scientia 5E56 film was used and developed for 4.5 minutes. At least two microradiograms were exposed for each pair of specimens. The microradiograms were photographed and prints made with an enlargement of 10.

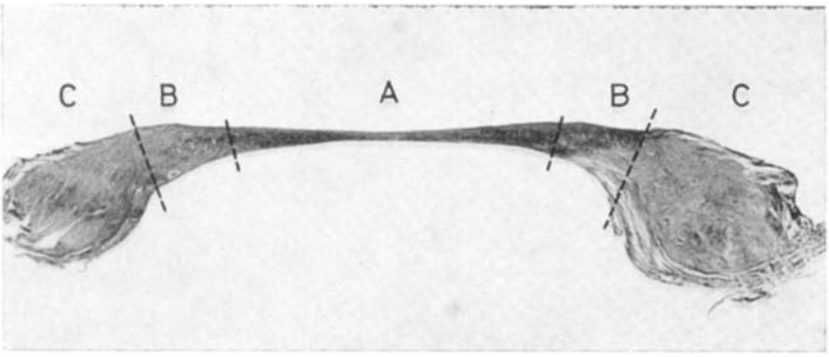

Fig. 1. Sagittal section through the mandibular disk from a 3-year old guinea pig. van Gieson. × 15.

Fig. 1. Sagittal section through the mandibular disk from a 3-year old packed collagen bundles run largely parallel to the surface of the joint, (B) an intermediate thicker portion were the direction of the bundles shows a greater variation, thus producing a three-dimensional network, and (C) a peripheral portion with still more loosely disposed bundles and an admixture of adipose tissue.

An analysis was performed by microscopic examination of the microradiograms, and by studying the enlargements to obtain an impression of the specimens and to locate any radiopaque areas.

Histologic examination. — After radiography the disks were fixed and imbedded in paraffin. Semiserial sagittal sections were cut and stained by the haematoxylin-eosin, van Gieson and von Kossa methods.

X-ray diffraction examination. — Representative radiopaque areas in the disks from one guinea pig in the oldest age group were examined by X-ray diffraction*.

Evaluation. — For the evaluation the disk was divided into two areas, one comprising the thin dense central and the thicker intermediate portion (A and B in figure 1) and the other the loose peripheral portion (C in the figure; see also *Öberg et al.,* 1965).

The presence of radiopaque areas in each disk was assessed by two of the authors together (simultaneous recording; *Markén,* 1962), without access to age data.

* The X-ray diffraction examination was kindly performed by Professor K-Å. Omnell, Department of Diagnostic Radiology, Royal School of Dentistry, Malmö, Sweden.

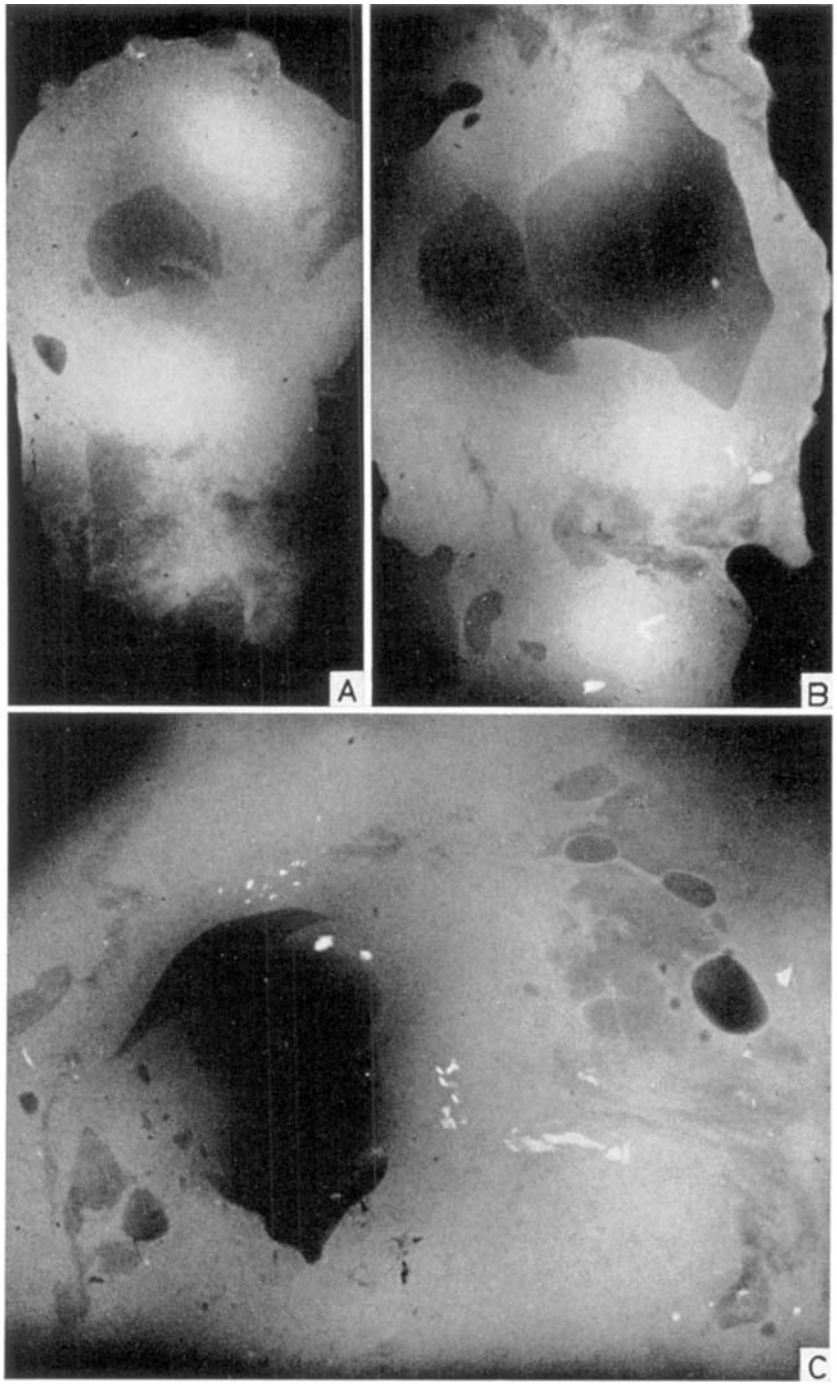

Fig. 2. Microradiogram of disk from (A) a one-month-old guinea pig; graded 0. (B) a 12-month old animal; graded 1. (C) a 4-year old animal; graded 2. Anterior at top in A and B, on left in C. × 10.

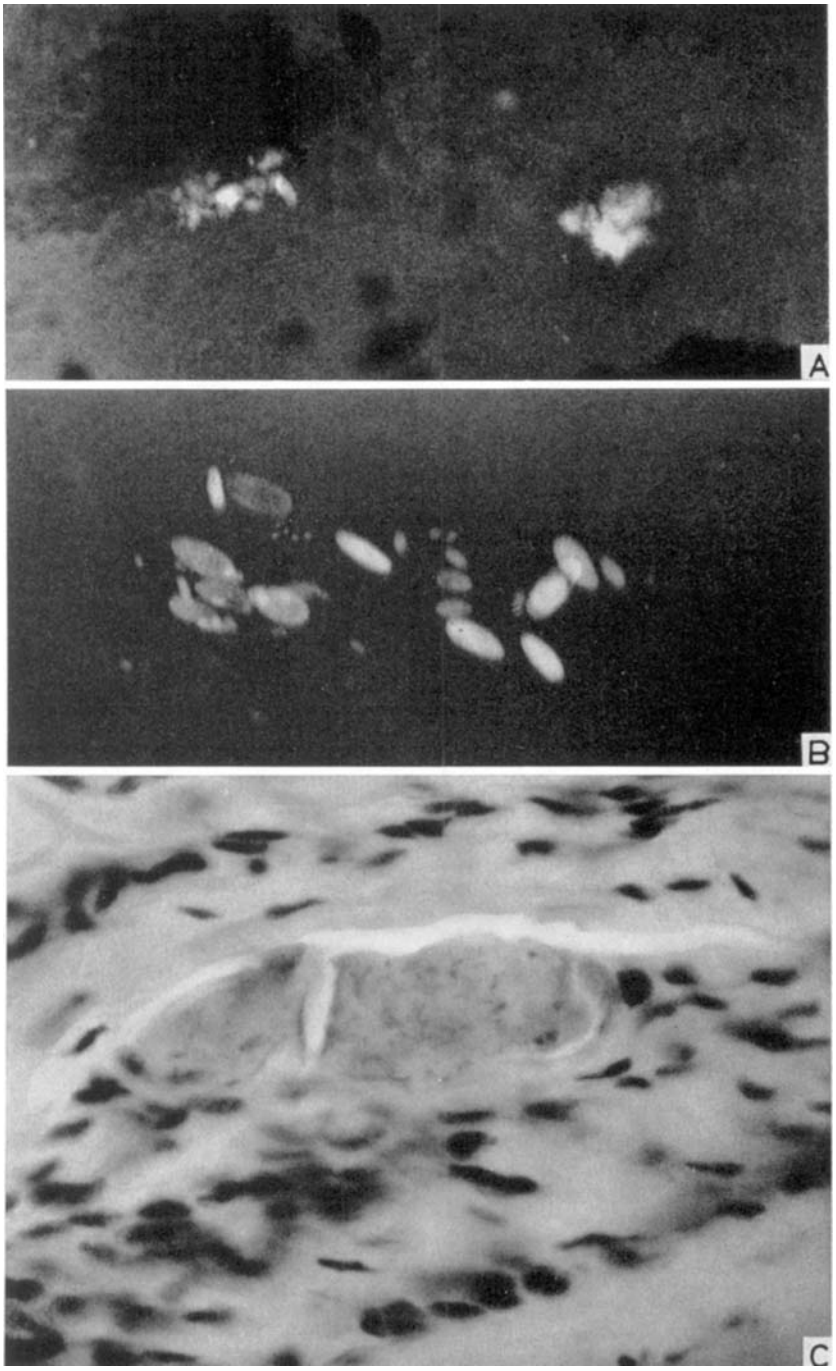

Fig. 3. Radiopaque areas under greater magnification: (A) Typical appearance of radiopaque areas. (B) Radiapaque areas observed in 2 of the oldest animals (from Fig. 2 C). × 60.

(C) Sagittal section through the central portion of the posterior part of the disk from an 18-month guinea pig; the section contains clearly demarcated homogeneous areas of basophile tissue, Haematoxylin-eosin. × 400.

The evaluations were made according to the following scale (Fig. 2):

0, Not more than a few small radiopaque areas

1, A few large and/or several small radiopaque areas

2, Numerous small and large radiopaque areas.

A radiopaque area is defined as a part of the microradiogram that is almost white and contrasts strongly with the surrounding tissue (Fig. 3).

RESULTS

Microradiographic observations

The radiopaque areas increased in number and extent with the age of the animal (Table II).

Table II

Distribution of mandibular disks according to the number and size of radiopaque areas represented by classes 0—2

	Central part			Peripheral part		
Class	0	1	2	0	1	2
Age group						
1 month	4	—	—	2	2	—
6 months	8	—	—	7	1	—
12	4	4	—	5	3	—
18	—	4	2	2	1	3
4 years	1	3	6	3	5	2

All the animals over 1 year displayed radiopaque areas in the disks. The areas were located chiefly in the loose peripheral (C) and intermediate (B) parts and in the peripheral part of the central portion (A). In the thinnest part of A, however, no radiopaque areas were observed with certainty.

The radiopaque areas varied in appearance and size (Fig. 2). Some of the smaller ones are shown in greater magnification in Figure 3. The formations in Figure 3 A with the irregular structure and shape are typical of most of the radiopaque areas. The

well demarcated elliptical areas in Figure 3 B were seen only in 2 of the oldest animals.

Histologic observations

In the histologic sections only a few homogenized areas were observed which were weakly stained blue by haematoxylin and brownish black with von Kossa stain. These were judged to be calcareous deposits, and probably corresponded to the radiopaque areas found in the microradiograms (Fig. 3 C).

X-ray diffraction examination

The radiopaque areas were found to consist of hydroxyapatite with the same crystallite size as for bone and dentine.

DISCUSSION

Representative radiopaque areas chosen by microradiography were found by X-ray diffraction to be calcareous deposits. Since only similar, light parts on the microradiographs contrasting strongly against the background were evaluated as radiopaque areas, it is highly probable that these were calcareous deposits, an interpretation that is supported by the histologic findings.

It is difficult to detect calcareous deposits in tissues by the usual histologic techniques. Being so time-consuming a procedure, serial sectioning can be used only on small series. Sectioning of calcified tissues, moreover, often presents considerable difficulty. Histologic staining methods for calcifications are also unreliable (*Tsaltas & Engfeldt,* 1963). These difficulties might account in some measure for the diversity of opinions on the presence of calcareous deposits in the human temporomandibular disk.

The soft X-ray technique used in the present study has several advantages over the usual histologic methods of detecting calcareous deposits. Most important, comparatively large volumes of tissue can be examined rapidly. In addition, a three-dimensional picture of the volume of the examined tissue can be obtained. The calcareous deposits and their position in relation to the other structures of the disk can be examined more easily and a better general impression is obtained. The soft X-ray technique

does not replace other examinations but can be followed by, for instance, serial sectioning or microchemical and microphysical examination of the areas that have yielded radiographic findings of interest.

The increase in the number of extent of radiopaque areas with the age of the animal is statistically significant for the central parts (A and B) of the disk. For the loose, peripheral part there is a similar tendency but 2 of the 4 disks examined from the 4-week guinea pigs were assigned to class 1. This may have been due to inclusion of bone or cartilage fragments in the dissection. The disks of the youngest animals were undoubtedly most difficult to dissect.

The present material displayed no changes associated with arthritis deformans. Nor did *Öberg*'s (1964) series of 270 newborn to 5-year-old guinea pigs contain any instance of such changes. There is therefore reason to deduce that the increase in calcareous deposits in the disk of the guinea pig with age is not a pathologic alteration but rather a normal ageing phenomenon.

It would seem to be of great clinical interest to apply the same technique to examine disks from human subjects for the presence of radiopaque areas. The present findings suggest that soft X-rays would bring to light calcareous deposits in a considerably greater number of disks than have histologic techniques. Studies at present being carried out on surgical and autopsy specimens would seem to bear out this supposition. It may then be possible to ascertain whether there is in general any connection between degenerative alterations in the disk, and pain and other symptoms of dysfunction in the temporomandibular joint.

SUMMARY

Disks from the temporomandibular joints of 18 guinea pigs ranging in age from 1 month to 4 years have been examined by contact microradiography and histologic techniques for the presence of radiopaque areas.

Representative radiopaque areas in the disks from a 4-year-old animal were found by X-ray diffraction to consist of hydroxyapatite, with the same size of crystallites as are found in bone and

dentine. It is therefore highly probable that the observed radiopaque areas were due to calcareous deposits.

The number and extent of the radiopaque areas (calcareous deposits) increased with age. The fact that all the animals over 1 year displayed such areas in the disks would suggest that they are linked with an ageing process. The microradiographic technique would seem to have advantages over the usual histologic methods for detecting calcareous deposits in the temporomandibular joint disk.

RÉSUMÉ

ETUDE MICRORADIOGRAPHIQUE DU MÉNISQUE TEMPORO-MANDIBULAIRE CHEZ LE COBAYE

Les auteurs ont examiné la présence de zones radio-opaques dans les ménisques temporo-mandibulaires de 18 cobayes âgés de 1 mois à 4 ans, au moyen de microradiographies par contact et de techniques histologiques.

Dans les ménisques d'un cobaye de 4 ans, on a constaté, par diffraction des rayons X, des zones radio-opaques représentatives consistant en hydroxy-apatite dont les cristallites se sont révélées être de même grandeur que celles qu'on trouve dans l'os et la dentine. C'est pourquoi il est extrêmement probable que les zones radio-opaques observées sont dues à des dépôts calcaires.

Le nombre et l'étendue des zones radio-opaques (dépôts calcaires) augmentaient avec l'âge. Le fait que, chez tous les animaux de plus d'un an, les ménisques aient présenté de telles zones semblait indiquer qu'elles seraient liées à un processus de vieillissement. La technique microradiographique semblerait présenter des avantages par rapport aux méthodes histologiques habituellement utilisées pour déceler les dépôts calcaires dans le ménisque de l'articulation temporo-mandibulaire.

ZUSAMMENFASSUNG

MIKRORADIOGRAPHISCHE UNTERSUCHUNGEN DER ZWISCHENSCHEIBEN DER KIEFERGELENKE VON MEERSCHWEINCHEN

Die Zwischenscheiben der Kiefergelenke (Discus articularis) von 18 Meerschweinchen in verschiedenen Altersgruppen von 1 Monate bis 4 Jahren, sind hinsichtlich des Vorkommens von Ver-

dichtungen mittels Kontaktmikroradiographie und histologischer Technik untersucht worden.

Mit Röntgendiffraktion haben sich repräsentative Verdichtungen der Zwischenscheiben eines 4-jährigen Tieres gezeigt, aus Hydroxyapatit mit derselben Kristallitgrösse wie bei Knochen und Dentin zu bestehen. Mit Hinsicht der angewandten Untersuchungstechnik ist es deshalb sehr glaublich, dass die registrierten Verdichtungen Verkalkungen sind.

Die Ergebnisse zeigen, dass Frekvenz und Umfassung der Verdichtungen (Verkalkungen) mit steigendem Alter der Tiere sich erhöhen. In samtlichen Tieren mehr als ein Jahr alt sind Verdichtungen (Verkalkungen) in den Gelenkzwischenscheiben entdeckt worden. Dieses wird als eine altersphysiologische Erscheinung gedeutet. In der Diskussion werden die Vorteile der mikroradiographischen Untersuchungstechnik gegen die gewöhnlichen histologischen Methoden pointiert, um Verkalkungen in den Geweben nachzuweisen.

REFERENCES

Bauer, W. H., 1941: Osteo-arthritis deformans of the temporomandibular joint. Am. J. Pathol., *17:* 129.

Blackwood, H. J. J., 1963: Arthritis of the mandibular joint. Brit. Dent. J., *115:* 317.

Boman, K., 1947: Temporomandibular joint arthrosis and its treatment by extirpation of the disc. Acta Chir. Scand., suppl. 118.

Macalister, A. D., 1954: A microscopic survey of the human temporomandibular joint. New Zealand Dent. J. *50:* 161.

Markén, K. E., 1962: Studies of deviations between observers in clinico-odontological recording. Transactions of the Royal Schools of Dentistry, Stockholm and Umeå, series *2:* 8.

Moffett, B. C., 1962: The temporomandibular joint. Published in Complete Denture Prosthodontics (Ed. J. J. Sharry). McGraw-Hill Book Co., New York/Toronto/London.

Öberg, T., 1964: Morphology, growth and matrix formation in the mandibular joint of the guinea pig. Transactions of the Royal Schools of Dentistry, Stockholm and Umeå, series *2:* 10.

Öberg, T., G. E. Carlsson, T. Ekberg & O. Hassler, 1965: Vascularization of the mandibular disk in the guinea pig. Odont. Revy *16:* 159.

Toller, P. A., 1961: The synovial apparatus and temporomandibular joint function. Brit. Dent. J., *111:* 355.

Tsaltas, T. T. & B. O. Engfeldt, 1963: A comparison of ultrasoft X-rays to histochemical methods for the detection of calcium in blood vessel wall. Acta Soc. Med. Upsaliensis, *68:* 147.

Address: *School of Dentistry,*
University of Umeå,
Umeå 6, Sweden.