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ELECTRON MICROSCOPY OF HUMAN CORONAL DENTINE
A METHODOLOGICAL STUDY WITH EMPHASIS ON THE "ASPIRATION" OF ODONTOBLAST NUCLEI

by

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INTRODUCTION

Pulp tissue and the odontoblasts present special problems with regard to fixation, because the soft tissue components are surrounded by a thick layer of calcified matrix which constitutes a barrier for the penetration of fixatives. The only direct soft tissue continuity between the pulp and the surrounding tissue is at the apical foramen. Apart from entering through this area, the fixatives will also penetrate calcified tissues (*Sorenson & Gatewood,* 1966), but it is a relatively slow process which may result in artefacts due to delayed fixation.

Several studies of the fine structure of the odontoblasts from various animals have been reported and some studies of the human odontoblasts have also been done (*Noble, Carmichael & Rankine,* 1962; *Frank,* 1966 a, b and 1968; *Arwill,* 1967). These authors were not dependent on getting odontoblasts from a definite area of the crown. However, in certain instances it is essential to be able to study specific areas of the pulp or the odontoblasts and the adjacent dentine. It has been demonstrated, for example, that the insertion of various filling materials produces localized changes in the pulp (e.g. *Langeland,* 1957) and in the dentine (e.g. *Mjör,* 1967b). Localized pulp/predentine/dentine alterations also occur in connection with

Received for publication, June 23, 1969.

attrition, caries and as a result of age. It was, therefore, considered essential to develop methods whereby ultrastructural studies of limited specific areas of coronal dentine could be performed.

Several difficulties have been encountered in getting good fixation of the odontoblasts from a definite experimental area (*Mjör & Furseth*, 1969), and these difficulties, as well as the procedures carried out to overcome them, will be reported and discussed in the present paper. One of these difficulties was the occurrence of aspirated odontoblast nuclei. This phenomenon will be given particular attention, since the ultrastructure of such aspirated nuclei has not been described previously.

MATERIALS AND METHODS

The material consisted of 53 specimens from 35 young human premolars which were extracted for orthodontic reasons (Table I). In the beginning of the investigation the roots were cut off by a diamond disc cooled with water, and the crowns were placed in the fixatives. In some instances an occlusal perforation was made in addition to cutting off the root. Both these methods resulted in poor fixation of the odontoblasts and will not be discussed in detail. In attempts to improve fixation four other procedures were then employed.

Table I.

Distribution ofmaterial and quality of fixation

Fixation procedure	Number of Teeth	Number of blocks (specimen)	Average score
1	8	11	1.3
2	7	11	1.5
3	14	22	2.4
4	6	9	2.5

1) Immediately after extraction, the root was cut off in the cervical region and the crown was cut into buccal and lingual halves with a diamond disc under constant water spray prior to fixation.

2) An occlusal perforation was made. A »local perfusion» was then attempted by injecting the fixative through the apical pulp by means of a syringe. Where the apical foramen was too narrow for a needle to be inserted, a small piece of the root was cut off. The »perfusion» lasted for approximately 10 minutes. The teeth were then left in the fixative for varying periods of time.
3) Prior to fixation the teeth were cut into small pieces with a diamond disc under constant water spray.
4) The root and the crown were separated by nippers and the crown further divided into smaller pieces by means of a knife and a hammer (*Frank*, 1969). The specimens were then placed in the fixative.

The fixatives used were either 2 % or 4 % formalin or 2.5 % glutaraldehyde; all buffered at neutral pH. Details regarding the fixatives and further details concerning methods have been described previously (*Furseth*, 1969). The fixation time varied from 10 min (*Frank*, 1966 a) to several days.

The specimens were postfixed in 1 % OsO_4 at neutral pH, dehydrated in graded solutions of acetone and embedded in Vestopal W (*Ryter & Kellenberger*, 1958). The blocks were trimmed, and 2—3 μ thick sections were cut for light microscopy and stained with 0.1 % toluidine blue in 0.067 M Na_2HPO_4 at pH 8.4. On the basis of the observations made from the toluidine blue stained sections, the quality of the fixation was classified as poor (grade 1), fair (grade 2), or good (grade 3) (Figs. 1—3).

The scoring was carried out by both authors independently. In certain specimens it was difficult to decide how to rate a particular section, thus intermediate values of 1.5 and 2.5 were assigned in these instances. By comparing the results obtained, it was found that the results never varied more than 0.5, and in these cases the higher value was used.

Ultrathin sections were then made of 15 specimens. The sections were stained with uranyl acetate and lead citrate (*Reynolds*, 1963) and examined in a Siemens Elmiskop Ia electron microscope operated at 80 kV.

RESULTS

The quality of fixation as scored in the toluidine blue stained sections is presented in Table I, and the criteria employed are illustrated in Figs. 1—3, which show toluidine blue stained sections with poor, fair and good fixation.

The correspondence between the fixation quality of the toluidine blue stained sections and the ultrathin sections was considered satisfactory. Fixa-

tion scored as 2.5 of 3 (fairly good or good) on the toluidine blue stained sections was found to give acceptable results for electron microscopy.

The structure of poorly fixed odontoblasts is shown in Fig. 5. The odontoblasts including the odontoblastic processes appear shrunken, and large empty spaces are found between the cells. However, the collagenous matrix of the predentine as well as the intertubular and peritubular matrix were well preserved. Fig. 6 gives an example of better fixation. The nuclei are well preserved, but the cytoplasm demonstrates some degree of shrinkage. Acceptable fixation of odontoblasts for electron microscopy is illustrated in Figs. 12, 15, 16 and 17. The nuclei, as well as the cytoplasm show fairly good preservation in these sections. Large intracytoplasmic vacuoles containing a fine fibrillar material and often a circular body of moderate electron density were frequently observed (Figs. 15, 17).

The first fixation procedure, i.e. cutting off the crown from the root and dividing the crown into buccal and lingual halves with a diamond disc, gave fair fixation of the odontoblasts in a few instances, but often the fixation was classified as poor (Table I). The »perfusion» method (procedure 2 — Table I) gave slightly better results, with fair and good fixation in a few instances, but with an average score of 1.5 (fair/poor fixation) it was not considered satisfactory.

The third procedure, to cut the tooth into small pieces with a diamond disc, was therefore attempted. This method gave fair or good fixation in most cases with an average score of 2.4 (Table I). But a complicating phenomenon was frequently encountered in that the odontoblast nuclei became displaced (aspirated) into the predentine or dentine (Figs. 4, 7—11). Most of the aspirated odontoblast nuclei had a homogeneous electron dense appearance, but some were electron lucent (Fig. 7). Canals were frequently observed within these nuclei. The canals (or invaginations) seemed to extend from the nuclear surface into the nucleus for varying distances (Figs. 8—10) and probably resulted from distortion of the nuclear membrane. It should be pointed out that the scoring in the third procedure was carried out in areas where aspiration had not occurred.

Numerous junctional complexes were observed between the odontoblasts, also in areas where aspiration might have occurred (Fig. 12). Intermediary and tight junctions were seen (Figs. 12, 13) and interdigitation of the cellular processes was noted in close connection with junctional complexes (Fig. 14).

The fourth procedure, cleavage of the tooth into smaller pieces by means of nippers and a knife and a hammer, gave an average score of 2.5 and was thus slightly superior to the third procedure (Table I).

DISCUSSION

In studies of localized areas of coronal dentine and pulp in animals, all the problems reported could probably be overcome by perfusion fixation. However, other fixation methods must be employed for human teeth, and the present study indicates that acceptable fixation methods for such studies are available.

The first two procedures employed in the present study did not result in acceptable fixation for electron microscopy, and should therefore be discarded.

It is apparently important to divide the teeth into sufficiently small pieces quickly, so that the fixative has easy access to the cells. This may be achieved by cutting the teeth with a diamond disc under water spray (procedure 3) or by cleavage with nippers, knives and a hammer (procedure 4). The third procedure employed is the best method for obtaining odontoblasts in one particular location, but it may result in displacement of odontoblast nuclei, and also in a disturbance of the content of the dentinal tubules (*Mjör*, 1967a). The method involving cleavage by nippers and knives is not hampered by the displacement of odontoblast nuclei, but whether a disturbance of the content of the tubules occurs has not been ascertained. However, this procedure is not as accurate as the third procedure. It therefore appears likely that a combination of procedures 3 and 4 may prove beneficial.

The mechanism involved in the displacement or aspiration of odontoblast nuclei is not known. Several conditions may induce this alteration, and the literature pertaining to this phenomenon has been reviewed by *Mjör* (1967a). It is evident that it may occur as a result of grinding both *in vivo* and *in vitro*, and it is important to prevent it during cavity preparation (e.g. *Langeland*, 1957; *Stanley & Swerdlow*, 1959). If occurring *in vivo*, it is considered to represent a permanent damage with an eventual disintegration of the aspirated nuclei (*Langeland*, 1957). The altered appearance of the aspirated nuclei observed in the present study supports the view that irrepairable damage has been done to the odontoblasts concerned. The aspiration of odontoblast nuclei *in vitro* is of interest in order to recognize it as an artefact on electron micrographs and thus be able to distinguish it from alterations caused by, for example, an experimental procedure.

The findings pertaining to the ultrastructure of odontoblasts in the present study, confirm evidence presented by others. The occurrence of junctional complexes between odontoblasts agrees with what has been reported earlier (e.g. *Frank*, 1966b). The presence of large mebrane-bound vacuoles or bodies near the nucleus similar to those observed in the present study has also been reported by *Frank* (1968), and he identified these bodies as

lysosomes. Since these vacuoles were extraordinarily large, however, the possibility that a swelling has occurred during preparation of the tissue should be considered.

SUMMARY

Four different procedures have been employed in order to evaluate the fixation of odontoblasts in the coronal portion of teeth. The material consisted of 53 specimens from 35 young human premolars. Sections from all the specimens were stained with toluidine blue. The quality of fixation was evaluated in the light microscope and graded as poor (1), fair (2), and good (3). Ultrathin sections were made from 15 specimens, and the correspondence between the fixation quality of the toluidine blue stained sections and the ultrathin sections was considered satisfactory. Two of the procedures, both involving a cutting of the teeth to the desired specimen size immediately after extraction, proved to result in satisfactory fixation. A complicating phenomenon was often encountered with one of these procedures, i.e. aspiration of odontoblast nuclei. These nuclei were homogeneously electron dense with canals extending from the nuclear surface into the nucleus for varying distances. The findings pertaining to the ultrastructure of unaffected odontoblasts, confirm evidence presented by others.

RÉSUMÉ

LA DENTINE CORONAIRE HUMAINE AU MICROSCOPE ÉLECTRONIQUE. ÉTUDE MÉTHODOLOGIQUE PORTANT EN PARTICULIER SUR "L'ASPIRATION" DES NOYAUX DES ODONTOBLASTES

Pour faire l'évaluation de la fixation des odontoblastes dans la partie coronaire des dents, 4 procédés différents ont été utilisés. Le matériel, consistait en 53 spécimens provenant de 35 jeunes prémolaires humaines. Des coupes provenant de tous les spécimens ont été colorées au bleu de toluidine. La qualité de la fixation a été évaluée au microscope optique et a reçu les mentions médiocre (1), acceptable (2), ou bonne (3). Des coupes ultra-minces ont été faites à partir de 15 des spécimens; la qualité de la fixation des coupes colorées au bleu de toluidine et celle des coupes ultra-minces se correspondaient de manière jugée satisfaisante. Une fixation satisfaisante a été obtenue avec deux des méthodes utilisées, nécessitant toutes deux que les dents soient sectionnées pour obtenir la grandeur du spécimen désirée immédiatement après l'extraction. Avec l'un des procédés, on rencontrait souvent un phénomène compliquant la situation: l'aspiration des noyaux des odonto-

blastes. Ces noyaux étaient uniformément opaques aux électrons, et présentaient des canaux s'étendant de la surface nucléaire vers l'intérieur du noyau sur une distance variable. Les résultats concernant l'ultrastructure des odontoblastes qui n'étaient pas touchés ont confirmé les preuves présentées par d'autres.

ZUSAMMENFASSUNG

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN VON KRONENDENTIN MENSCHLICHER ZÄHNE

EINE METHODOLOGISCHE STUDIE MIT BESONDEREM GEWICHT AUF DIE "ASPIRIERUNG" DER ODONTOBLASTKERNE

Um die Odontoblasten in den Kronen der Zähne zu fixieren wurden vier verschiedene Verfahren verwendet. Das Material bestand aus 53 Preparaten von 35 jungen Premolaren. Von allen Preparaten wurden Schnitte mit Toluidinblau gefärbt. Die Qualität der Fixierung wurde im Lichtmikroskop als schlecht (1), mittelmässig (2) und gut (3) abgestuft. Von 15 Preparaten wurden ultradünne Schnitte hergestellt. Der Zusammenhang zwischen den mit Toluidinblau gefärbten Schnitten und den ultradünnen Schnitten wurde als befriedigend beurteilt. In zwei von den Methoden wurden die Zähne sofort nach der Extraktion in die gewünschte Preparatgrösse geschnitten, und die Fixierung war befriedigend. In einer von diesen Methoden wurde oftmals eine Aspirierung der Odontoblastkerne als eine komplizierte Erscheinung gefunden. Diese Kerne waren homogen, elektronendicht und mit Kanälen versehen, die von der Kernoberfläche ungleich tief in den Kern hineinreichten. Was die Ultrastruktur der unveränderten Odontoblasten betrifft, so ist dieses von anderen Authoren beobachtet worden.

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Plate 1.

Figs. 1—3. Photomicrographs of undecalcified, toluidine blue stained sections illustrating various qualities or grades of fixation of odontoblasts. Fig. 1 is an example of poor fixation (grade 1). The nuclei and cytoplasm of the odontoblasts are markedly shrunken. Fig. 2 illustrates fair fixation (grade 2), and the same artefacts are present, but less pronounced than in Fig. 1. Fig. 3 shows good fixation (grade 3). The nuclei are lightly stained with distinct nucleoli and the cytoplasm appears not to have shrunk. Odontoblasts (OB); predentine (PD); dentine (D). ×640.

Fig. 4. Photomicrograph of undecalcified, toluidine blue stained section showing aspirated odontoblast nuclei in the predentine (PD) and dentine (D). ×640.

Plate 2.

Fig. 5. Electron micrograph of coronal dentine illustrating poor fixation (grade 1 on toluidine blue stained section). The odontoblasts (OB) including the odontoblastic processes, appear shrunken and large empty spaces are found between the cells. Predentine (PD); dentine (D). ×3,300.

Fig. 6. Electron micrograph of coronal dentine showing fairly good fixation of the odontoblasts (grade 2.5) on toluidine blue stained section. The nuclei (N) are well preserved, while the cytoplasm appears vacuolated and some degree of shrinkage can be noted. Predentine (PD). ×3,600.

Plate 3.

Fig. 7. Electron micrograph of coronal dentine showing several aspirated odontoblast nuclei (AON) in the predentine (PD). Fixation was classified as grade 2.5 on toluidine blue stained sections in areas where aspiration had not occurred. The area pulpal to the predentine is devoid of nuclei in this particular region. Most of the aspirated odontoblast nuclei appear electron dense, but one of the aspirated odontoblasts (arrow) appears more electron lucent. ×3,300.

Fig. 8. Electron micrograph from the predentine (PD) showing parts of three dentinal tubules, two of which contain electron dense, aspirated odontoblast nuclei (AON). Higher magnification of one of the aspirated nuclei is shown in Fig. 9. ×22,800.

Plate 4.

Fig. 9. Higher magnification of one of the aspirated odontoblast nuclei (AON) shown in Fig. 8. The nucleus is quite dense, and canals are observed from the surface into the nucleus. The canals are possibly caused by folding and compression of the nuclear membrane. ×66,500.

Fig. 10. Electron micrograph showing aspirated odontoblast nucleus (AON) in the predentine (PD). The nucleus has a homogeneous electron dense appearance and longitudinally as well as cross-sectioned canals can be seen. ×21,000.

Fig. 11. Electron micrograph showing longitudinally sectioned aspirated odontoblast nucleus (AON) at the predentine (PD)/dentine (D) interface. Note the homogeneous electron dense texture and the electron lucent canals in the nucleus. ×22,800.

Plate 5.

Fig. 12. Electron micrograph showing aspirated odontoblast nucleus (AON) in the predentine. Fixation was classified as grade 2.5 on toluidine blue stained sections. Several cross-sectioned odontoblasts partly connected with junctional complexes (arrows) can be noted. Higher magnification of area indicated with double arrows is shown in Fig. 13. ×6,000.

Fig. 13. Higher magnification of junctional complex indicated with double arrows in Fig. 12. The junctional complex is partly made up of a tight junction (TJ) and partly of an intermediary junction (IJ). ×3,200.

Fig. 14. Electron micrograph showing two odontoblasts (OB) connected with junctional complex. Note close interdigitation of cellular processes (arrow). ×32,000.

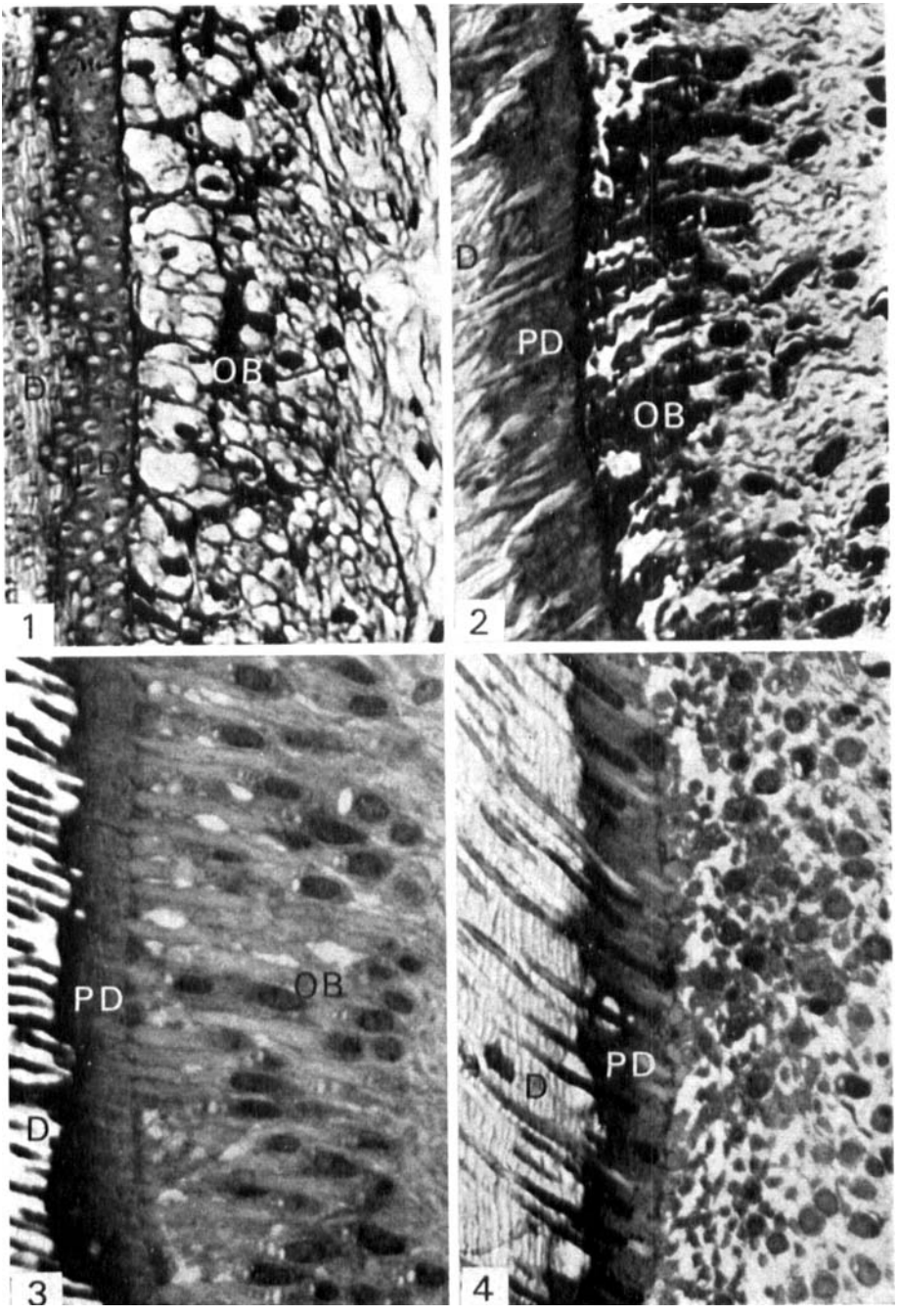
Plate 6.

Fig. 15. Electron micrograph showing what is considered to be fairly good fixation of the odontoblasts (OB) (grade 2.5 on toluidine blue stained section). Note several large vacuoles close to the nuelei. Higher magnification in Fig. 17. ×3,000.

Fig. 16. Electron micrograph showing good fixation of the odontoblasts (OB) (grade 3 on toluidine blue stained section). Predentine (PD); odontoblastic process (OP). ×6,000.

Plate 7.

Fig. 17. Higher magnification of the odontoblast shown in upper left corner in Fig. 15. Mitochondria (M), Golgi apparatus (G), and endoplasmic reticulum (ER) are well preserved. Large vacuole (V) containing scattered, fine fibrillar material and a homogeneous circular body of moderate electron density is found in the cytoplasm close to the nucleus. ×22,400.



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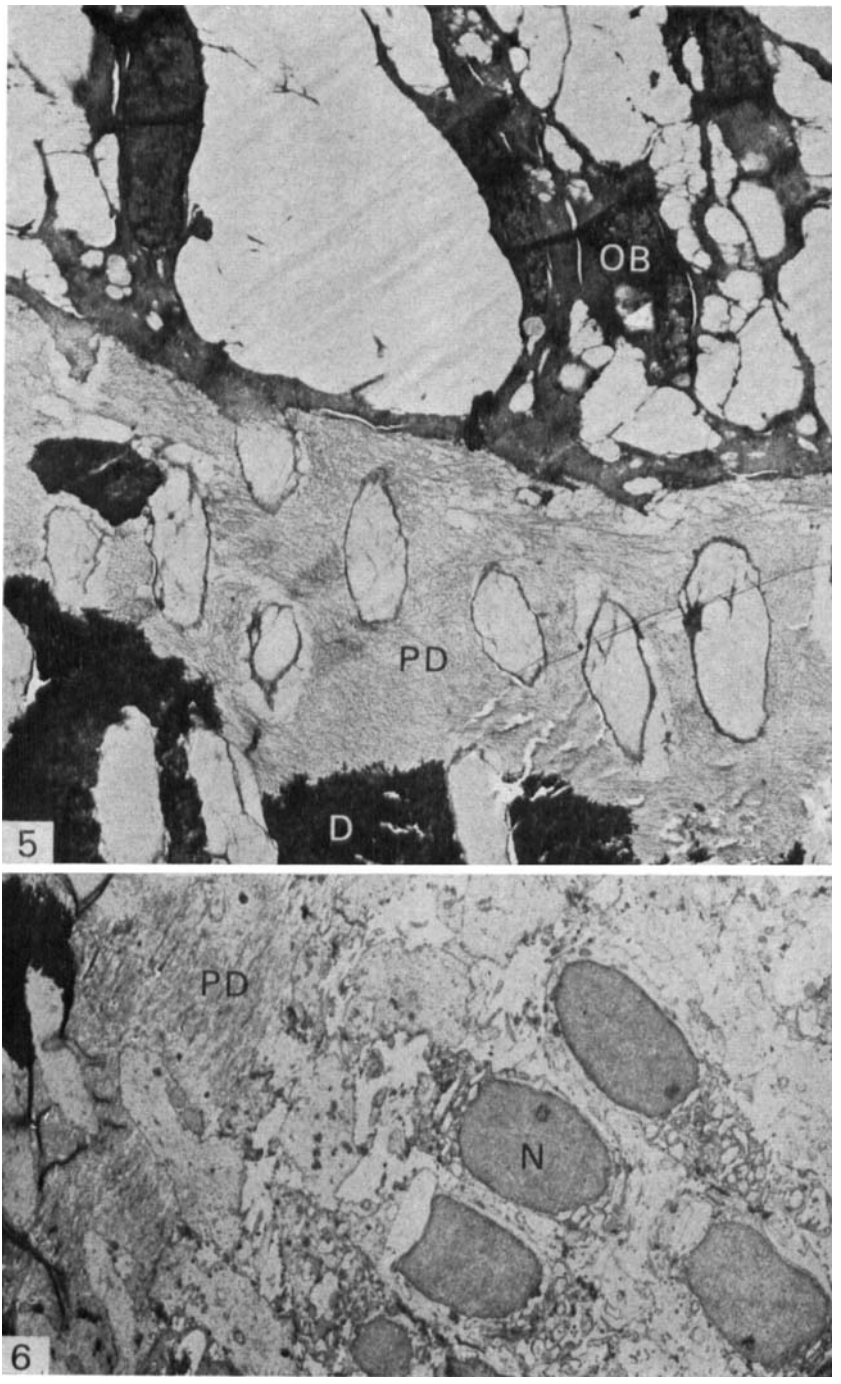

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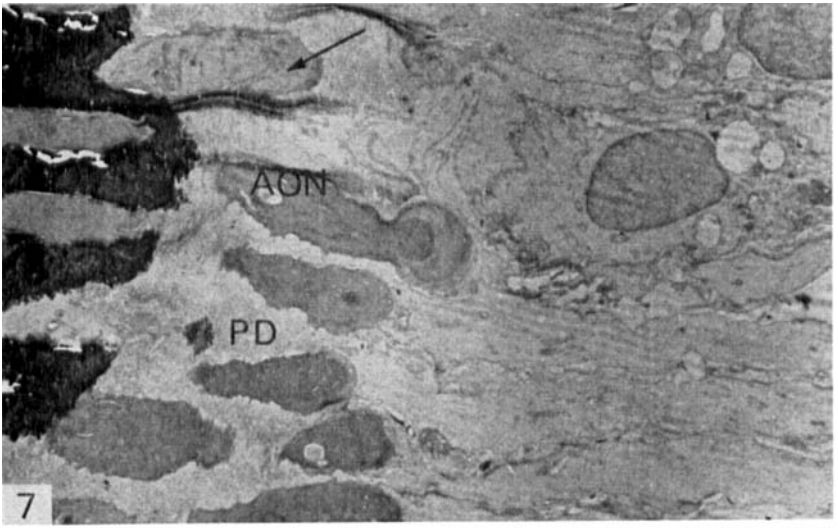

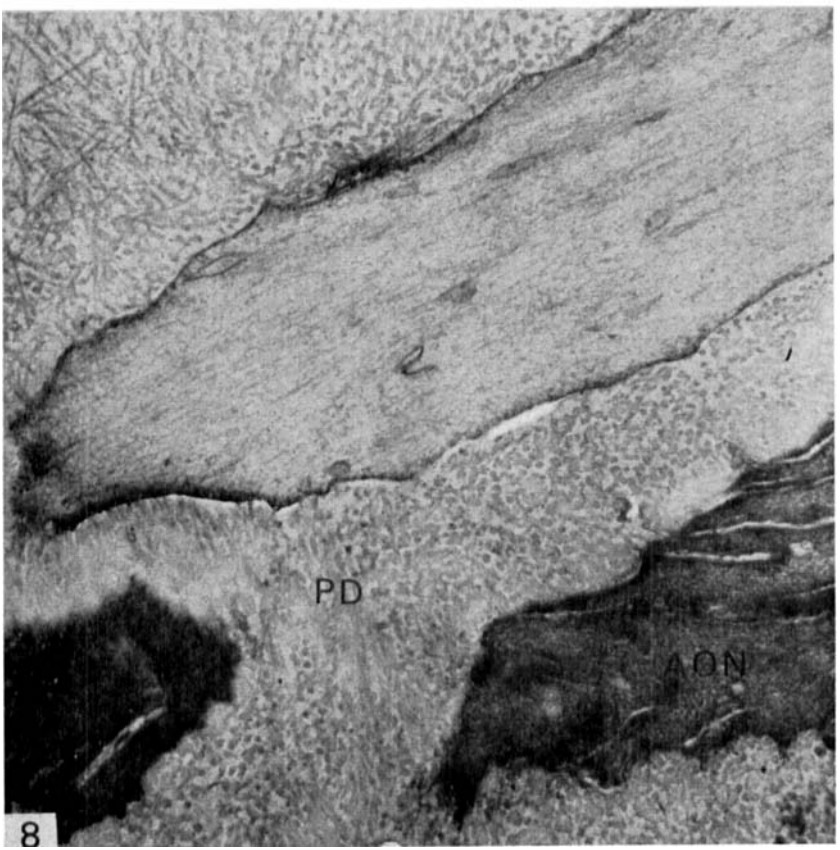

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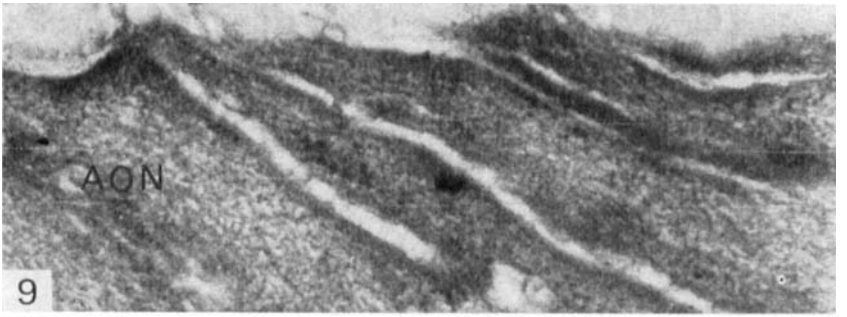

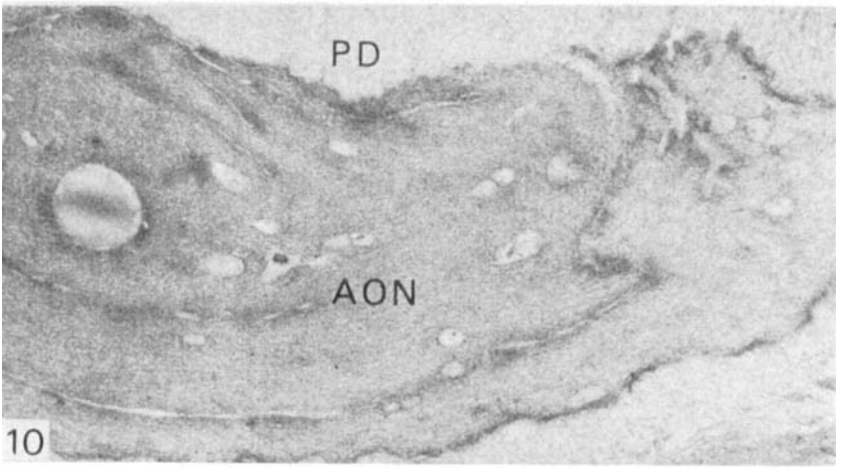

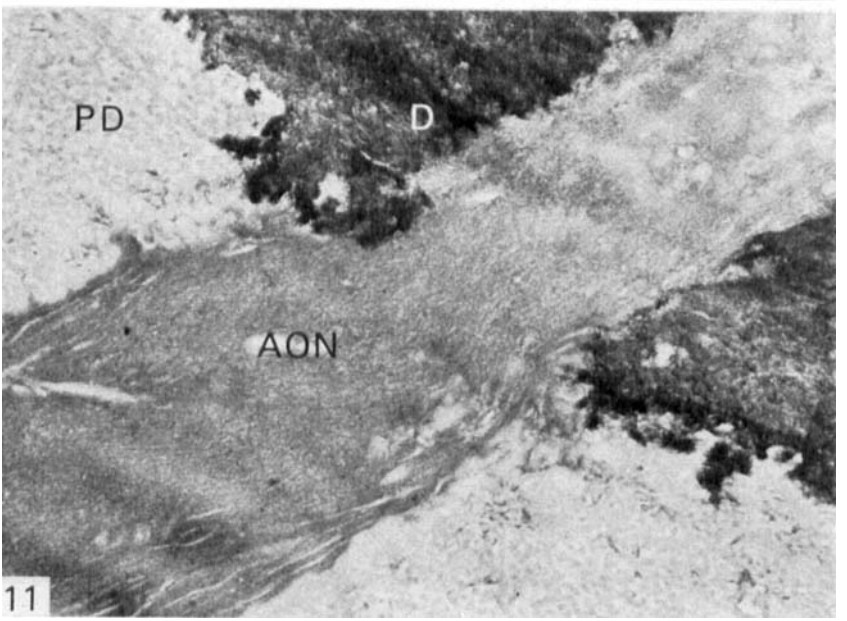

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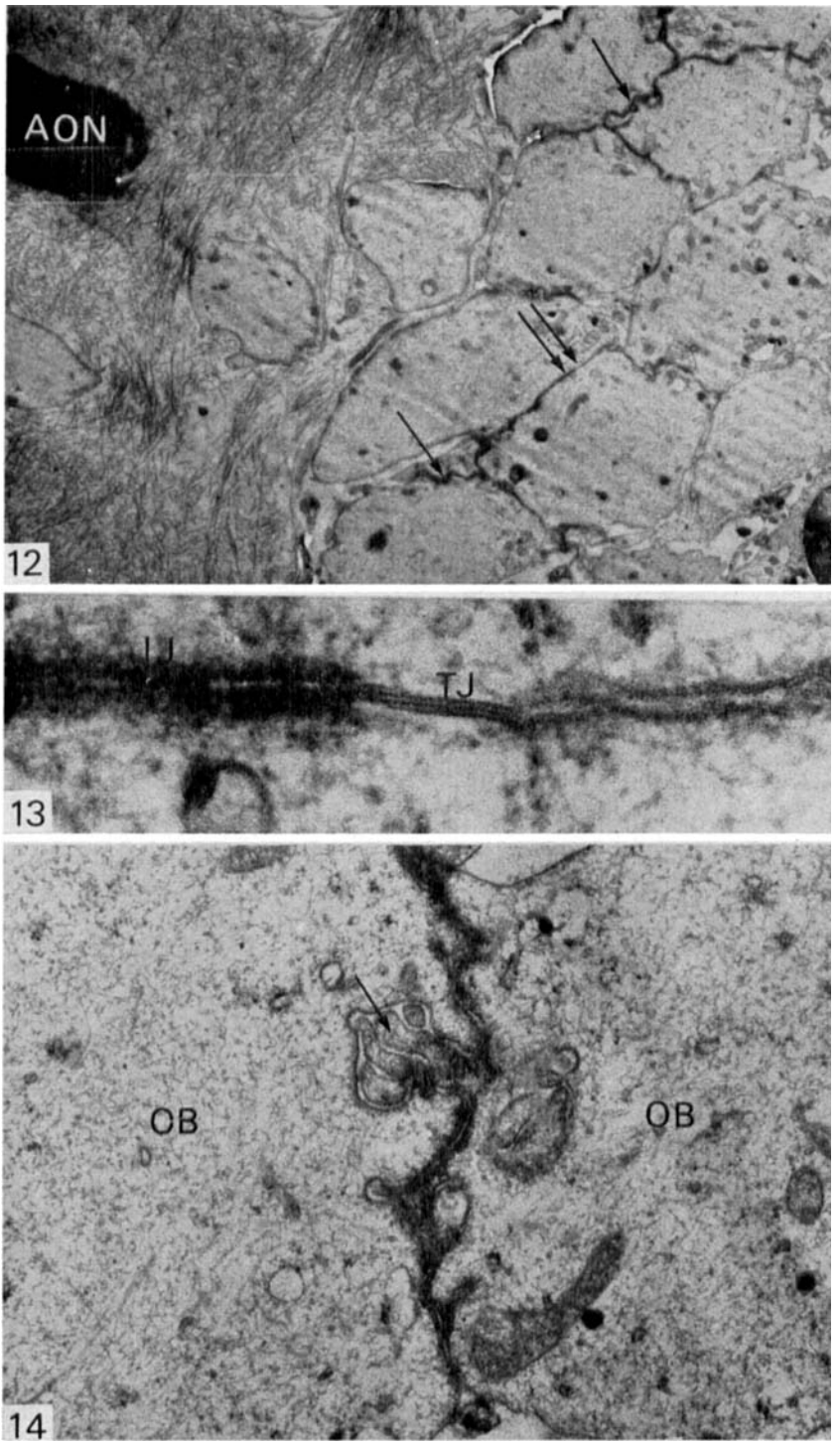

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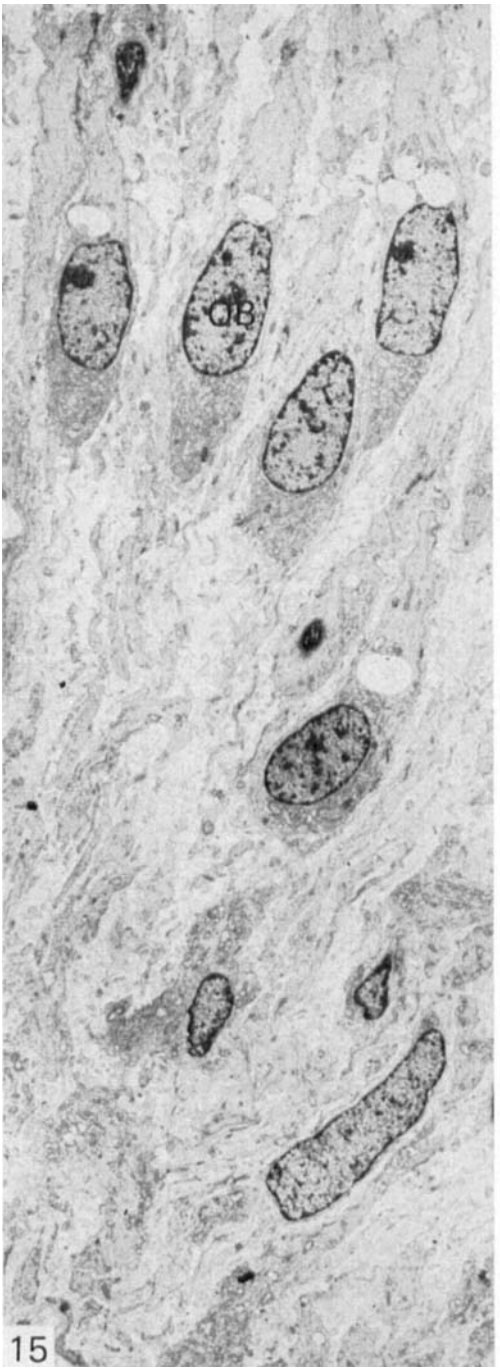

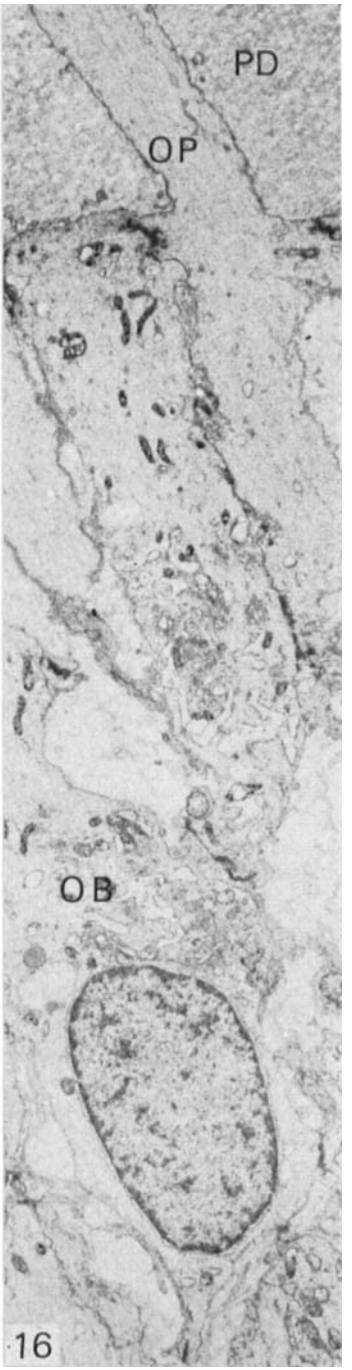

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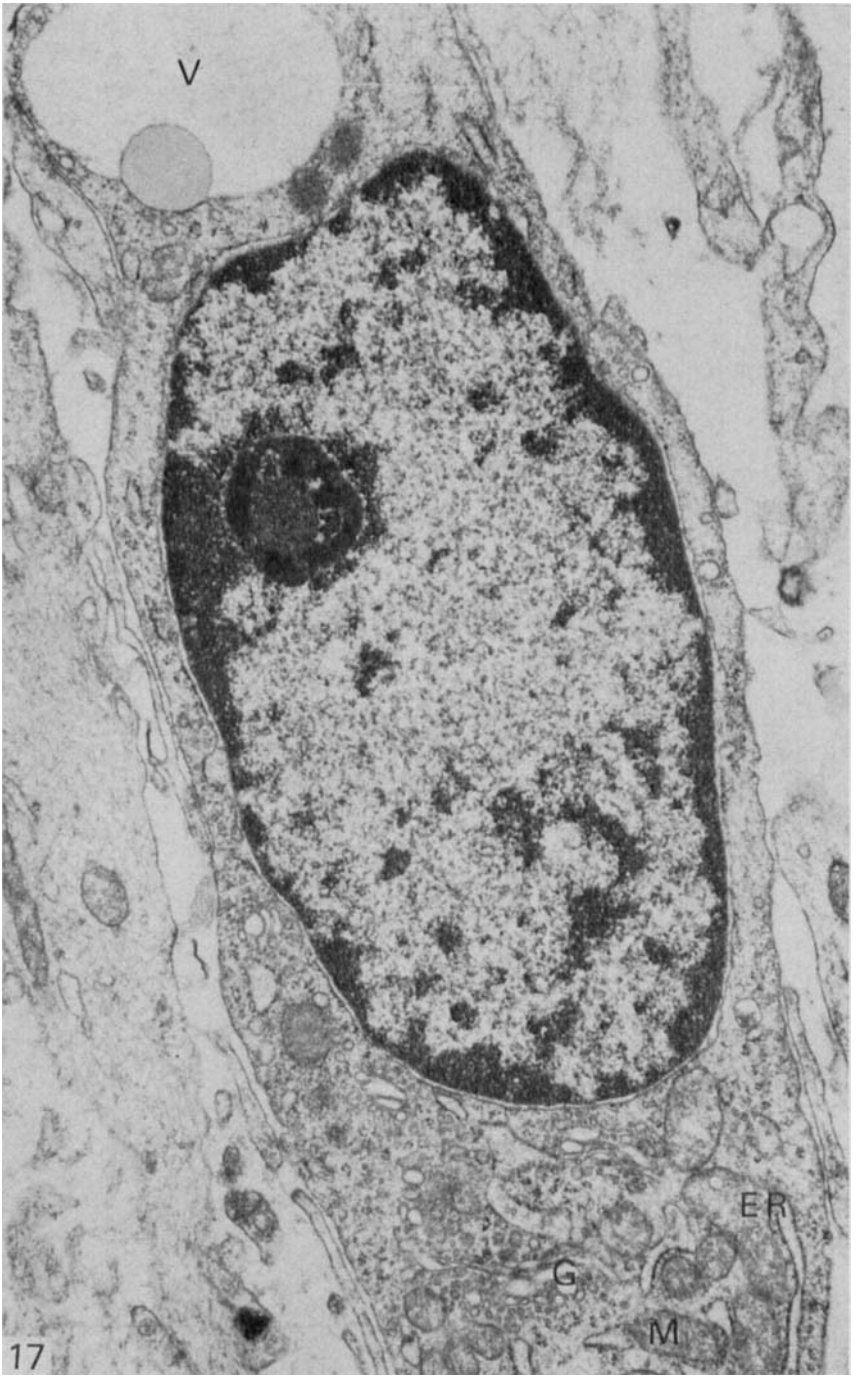

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