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OBSERVATIONS ON THE PERACETIC ACID-ALDEHYDE FUCHSIN (OXYTALAN) POSITIVE TISSUE ELEMENTS IN THE PERIODONTIUM

by

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In 1958 *Fullmer & Lillie* described a fibre component of connective tissue which was resistant to acid hydrolysis and could be demonstrated in the periodontal membrane by a peracetic-aldehyde fuchsin staining system. Primarily, it was suggested that these acid "oxytalan" fibres could be a special form of collagen which developed in areas of stress. Further investigation (*Fullmer & Lillie* 1959), however, produced evidence that they were distinct from collagenous and reticulin fibres, as well as from mature elastic fibres. Series of histochemical tests including enzyme digestion and chemical treatment have failed to identify positively the nature of the oxytalan fibres. On the evidence that the stainable material associated with the fibrous structures was digestible by lysozyme and testicular hyaluronidase after peracetic acid oxidation, it was suggested that this component was a mucopolysaccharide or a mucoprotein of a special type found in elastic and "oxytalan" fibres.

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Subsequent publications examined the distribution and development of these fibres in the periodontium of rodents, monkeys and man; in the developing and mature state as well as in periodontal disease, (*Fullmer* 1961 and 1962); in ameloblastomas (*Fisher & Fullmer* 1962); in dental granulomas and radicular cysts (*Fullmer* 1960).

The findings and conclusions drawn from all these papers may be summarized by stating that these authors consider:

1. Oxytalan fibres represent fibres of a special mucopolysaccharide nature.
2. They may represent immature elastic fibres or attempts by the body to form these.
3. They occur predominantly in areas of tissue breakdown and repair, and could represent early degenerative changes in collagen.
4. They are inserted into cementum or, in the case of cyst walls and ameloblastomas, may run parallel to the collagen fibre components.

The present paper deals with our experience of the occurrence of peracetic acid-aldehyde fuchsin positive material in the periodontal membrane and its demonstration by the technique of *Fullmer & Lillie* (1958) and our modifications.

MATERIAL AND METHODS

The teeth and supporting tissues of one normal adult dog, three normal adult monkeys (Rhesus maccacus), and several human teeth with their supporting tissues were fixed in 10 per cent buffered formalin and decalcified in 5 per cent formic acid. Paraffin blocks and sections were prepared in a routine manner.

Sections were stained in aldehyde fuchsin after pre-treatment with peracetic acid according to the technique described by *Fullmer & Lillie* (1958) with the recommended counterstains. Four different brands of basic fuchsin were used.

Other sections were stained according to the following modified technique:

1. Greenspan's peracetic acid (*Lillie* 1954) — 60 minutes
2. Wash
3. Aldehyde fuchsin — 15 minutes.

The aldehyde fuchsin was prepared from any of four brands as follows:

5 gm basic fuchsin
100 ml absolute ethyl alcohol
4 ml paraldehyde
2 ml concentrated hydrochloric acid.

The prepared mixture was allowed to stand at room temperature for 12 hours and then stored in a refrigerator and filtered before use. It was found to be an advantage to reduce the background stain by treatment for 2 minutes in 4 per cent hydrochloric acid in 70 per cent alcohol. No counterstain was used.

Further sections were stained in orcinol-new fuchsin by the method of *Fullmer & Lillie* (1956).

Some sections of each type were stained for peripheral nerves by the method of *Fearnhead & Linder* (1956).

The sections were examined in both polarized and non-polarized light.

RESULTS

Peracetic Acid-Aldehyde Fuchsin

Using the technique of *Fullmer & Lillie* (1958) it was found that oxytalan fibres could be demonstrated only haphazardly. Varying brands of basic fuchsin yielded no better results.

Omitting the counterstains revealed the presence of a weak overall purple background stain, which had apparently been either bleached or removed by the acid of the Halmi counter stain of the full technique (see Table I). Staining in aldehyde fuchsin without the peracetic acid oxidation produced a similar background stain. Increasing the oxidation time served only to increase this background stain; this, however, could be totally removed by brief exposure to acid alcohol (Figs. 1 and 2), whilst increasing the time in aldehyde fuchsin served no useful purpose.

COLOUR PLATE

Fig. 1. Decalcified tangential section through the apical area of a maxillary molar of a rhesus monkey stained with peracetic acid-aldehyde fuchsin. Note the heavily stained axial fibres against a dark background.

Fig. 2. Similar section to fig. 1 differentiated in 4 % acid alcohol for 60 secs. Note the absence of background stain and the persistence of the oxytalan positive fibres.

Fig. 3. Similar section as in figs. 1 and 2 but stained by Fearnhead-Linder silver technique for nerve fibres. Note the argyrophilic axial fibres.

Fig. 4. Decalcified section through the maxillary molar periodontium of a Rhesus monkey stained by peracetic acid-aldehyde fuchsin-Halmi sequence, showing fibres emerging from bone marrow spaces into an axial course within the periodontal membrane.

Fig. 5. Another area of the specimen shown in fig. 4, showing the axial fibres interlacing the principal fibres of the periodontal membrane. Some oxytalan fibres emerge from the cementum.

Fig. 6. Decalcified section through the maxillary premolar periodontium of a Rhesus monkey stained by peracetic acid-aldehyde fuchsin-Halmi sequence, showing oxytalan fibres emerging from the cementum.

Fig. 7. Decalcified section through the maxillary premolar periodontium of a Rhesus monkey silver impregnated by the Fearnhead-Linder technique showing argyrophilic axial fibres within the periodontium.

Fig. 8. Another portion of the section shown in fig. 7 showing a high power view of one argyrophilic fibre emerging from the cementum.

Fig. 9. Decalcified section through the maxillary premolar periodontium of a rhesus monkey stained by Peracetic acid-aldehyde fuchsin acid alcohol sequence showing fine branching oxytalan fibres some arising from the cementum.

Fig. 10. Decalcified section through the pulp of a molar tooth of a Rhesus monkey stained by peracetic acid-aldehyde fuchsin-Halmi sequence showing oxytalan positive fibres.

Fig. 11. Section through striated muscle from a dog showing an oxytalan positive muscle end plate.

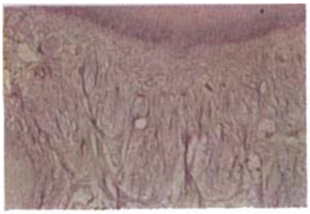

FIG. 1

FIG. 2

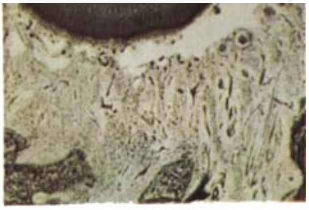

FIG. 3

FIG. 4

FIG. 5

FIG. 6

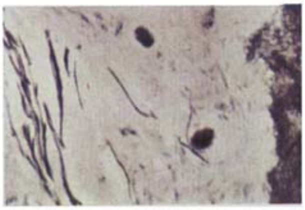

FIG. 7

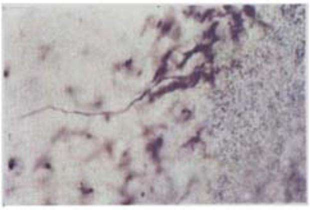

FIG. 8

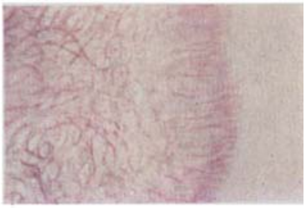

FIG. 9

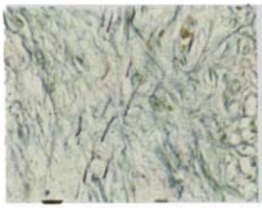

FIG. 10

FIG. 11

A. A low level of intensity in bone and dentine matrices, in basement membranes and intercellularly both in keratinizing and non-keratinizing surface epithelia. Also in the epithelial rests within the periodontal membrane.

B. A greater intensity was found in the incremental lines of bone; in the cementum and predentine and within dentinal tubules.

C. A very high staining intensity was limited to an oxytalan positive material in the form of solid, individual fibrous structures orientated parallel to the axis of the tooth (Figs. 4 and 5). These axial fibres described an interlacing course between the principal fibre bundles and could be observed to be up to 2 mm in length.

   One end of each fibre emerged with the major blood vessels from the alveolar bone (Fig. 4). Many of these fibres were seen to branch with some of the branches having free terminations between the principal fibre bundles. Other branches appeared to arise peripherally and where they could be traced, described a course from a commencement in the cementum to where they joined the axial fibre. These branches of the main axial fibres were thinner and in some specimens arose from a fine network of similar fibres adjacent to and emerging from the cementum (Figs. 6 and 9). These peripheral branching fibres were not observed emerging from the alveolar bone.

Fibres of a similar size as the peripheral branching fibres and with the same staining characteristics could be observed in the connective tissue in general and in the corium of both free and attached gingiva.

Orcinol-New Fuchsin

The identification of the oxytalan positive fibres with elastic fibres was ruled out by the parallel orcinol-new fuchsin stain, performed on alternate sections which, though demonstrating abundant elastic fibres in the sub-epithelial connective tissue of the attached gingiva and larger blood vessels, failed to stain any structure within the periodontal membrane.

Silver Impregnation

The Fearnhead-Linder silver impregnation for peripheral nerves produced a distribution of silver stained fibres similar to the distribution of the oxytalan positive fibres (Figs. 3, 7, 8). The axial fibres (Fig. 7) were stained deeply and in those sections previously stained with the oxytalan technique these fibres were clearly identical with those seen before. The fine network of small fibres adjacent to and emerging from cementum could, however, only be seen with difficulty (Fig. 8).

DISCUSSION

This investigation has indicated that there are fibrous structures within the periodontium which stain somewhat selectively with peracetic acid-aldehyde fuchsin; the axial fibres, and the peripheral branching fibres both of which are found in the periodontal membrane and elsewhere.

Fullmer (1958, 1959, 1961 and 1962), *Fischer & Fullmer* (1962) and *Fullmer & Witte* (1962) suggested that these oxytalan positive structures are related to some component of fibres specifically found in areas of stress, degeneration and repair. The present findings, on the other hand, would indicate that this material is a normal component of many tissues.

These oxytalan positive fibres are not related to collagen as evidenced by their staining characteristics and weak birefringence in polarized light. The fact that the axial fibres branch could indicate that there may be some relationship between them and elastic fibres. However, the chemical and physical characteristics of the fibres in question indicate that they are not composed of elastin, and their course does not conform to any known component of the attachment apparatus. Their scarcity, in any case, precludes any tooth-supporting function. The origin and the course described by the axial fibres, their morphology and argyrophilic properties suggest that they are neural elements. Moreover, similar fibres can be demonstrated in the pulp (Fig. 10), and further support for this hypothesis comes from the finding of oxytalan positive motor endplates in striated muscle (Fig. 11). Their distribution within the periodontal membrane and the limited control procedure with orcinol-new fuchsin and testicular hyaluronidase seems to confirm the conclusion that these fibres are the oxytalan fibres described by *Fullmer* and his co-workers.

The identity of the peripheral branching fibres with small peripheral nerves is open to further investigation since silver techniques make the tracing of fine nerves difficult due to the inherent granularity of the silver deposit. However, it must be pointed out that it is possible, both in silver-impregnated and oxytalan preparations to see the peripheral branching fibres entering the axial fibre bundles so that the present authors are in little doubt as to the continuity of these structures.

It is pertinent to note that the morphology of the neural elements within the periodontal membrane as described by *Bernick* (1957) corresponds closely to the course described by the oxytalan positive and argyrophylic fibres of the present study.

SUMMARY

Decalcified sections of the teeth and periodontium of monkey, dog and man were stained by peracetic acid-aldehyde fuchsin, orcinol-new fuchsin and impregnated with silver and viewed in polarized and non-polarized light.

Both the silver and the peracetic acid-aldehyde fuchsin stained morphologically identical fibres. Orcinol-new fuchsin failed to stain these structures. Attention is drawn to the similarity between oxytalan fibres and nerve elements.

RÉSUMÉ

OBSERVATIONS SUR LES ÉLÉMENTS POSITIFS À L'ACIDE PERACÉTIQUE-FUCHSINE ALDÉHYDE (OXYTALAN) DANS LES TISSUS DU PARODONTE

Des coupes décalcifiées des dents et du parodonte de singe, de chien et d'homme ont été colorées par l'acide peracétique-fuchsine aldéhyde, par l'orcine-fuchsine nouvelle, et par imprégnation à l'argent, et examinées en lumière polarisée et en lumière non polarisée.

L'argent et l'acide peracétique-fuchsine aldéhyde coloraient tous deux des fibres identiques du point de vue morphologique. L'orcine-fuchsine nouvelle n'a pu colorer ces tissus. Les auteurs attirent l'attention sur la ressemblance entre les fibres positifs à l'acide peracétique-fuchsine aldéhyde (oxytalan) et les éléments nerveux.

ZUSAMMENFASSUNG

BEOBACHTUNG VON PERESSIGSÄURE-ALDEHYD FUCHSIN (OXYTALAN) POSITIVEN GEWEBSELEMENTEN IM PARODONTIUM

Decalcifizierte Schnitte der Zähne und des Parodontiums von Affen, Hunden und Menschen wurden mit Peressigsäure-Aldehyd und "Orcinol-new Fuchsin" gefärbt und mit Silber imprägniert. Die Präparate wurden in polarisiertem und nicht-polarisiertem Licht betrachtet.

Sowohl das Silber als das Peressigsäure-Aldehyd Fuchsin färbten morphologisch identische Fasern. "Orcinol-new Fuchsin" färbte diese Strukturen nicht. Man bemerke die Ähnlichkeit zwischen Oxytalanfasern und Nervenelementen.

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