THE FINE STRUCTURE OF THE OXYTALAN FIBER

G. G. CARMICHAEL and H. M. FULLMER

From the National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland. Dr. Carmichael's present address is the Institute of Medical and Veterinary Science, Adelaide, South Australia

ABSTRACT

Maraglas-embedded sections of periodontal membranes around continually growing incisors of Sprague-Dawley rats fixed consecutively in cold glutaraldehyde and Palade's 1% buffered osmium tetroxide were examined under the electron microscope for oxytalan connective tissue fibers. Oxytalan fibers were noted to consist of bundles of filaments approximately 150 A in diameter with an interfilamentous amorphous substance of approximately the same diameter. A periodicity of fibrillar elements was not obvious.

INTRODUCTION

The oxytalan fiber is a connective tissue element that has been described by Fullmer and Lillie (1958), and has been most extensively studied in the periodontal membrane. The fibers also occur in tendon, ligament, vascular adventitia, nerve, and around the skin appendages in the dermis (Fullmer, 1959), and have been shown in some pathological situations (Fullmer, 1960 a; Fisher and Fullmer, 1962). The studies of Kohl and Zander (1962) and Rannie (1963) confirm the status of the oxytalan fiber as a constituent of the connective tissue.

The name "oxytalan" was derived from the fact that the fibers withstand formic acid degradation. Up to the present time they have remained an entity that is demonstrable only by histochemical methods. Treatment by a strong oxidizing agent is a prerequisite, and peracetic acid was most commonly used (Fullmer and Lillie, 1958): however, more recently, a monopersulfate compound (Oxone, E. L. DuPont De Nemours & Co., Inc., Wilmington, Delaware) has been used by Rannie (1963), with more satisfactory technical results. After the stage of oxidation, the fibers are seen to best advantage by staining with Gomori's aldehyde fuchsin, and orcein and resorcin fuchsin show up some fibers after preoxidation. Staining

with either Verhoeff's ferric chloride hematoxylin or orcinol-new fuchsin, after oxidation, does not reveal the oxytalan fiber.

Histochemical and morphological studies, at the magnifications of light microscopy, provide some evidence for speculation of a relationship to elastic fibers and indicate that the oxytalan fiber should be placed in the same category as elastic fibers, and that also there is more than one structural component. Thus, the staining reaction is abolished readily by β-glucuronidase, and by elastase after the stage of oxidation (Fullmer, 1960 b; Fullmer and Lillie, 1958).

MATERIALS AND METHODS

Electron Microscopy

Female Sprague-Dawley rats, aged 8 to 10 months, were quickly sacrificed with chloroform, and the jaws were placed in cold buffered glutaraldehyde (6.0%). The incisor teeth were sawn with a fine fret saw in a buccolingual direction, immediately anterior to the molar teeth. They were then placed in fresh cold 5% glutaraldehyde, and allowed to fix for at least 4 hr. When the soft tissues appeared to be fixed, the teeth were held in a vise and the enamel on the labial surface was sawn off. The re-
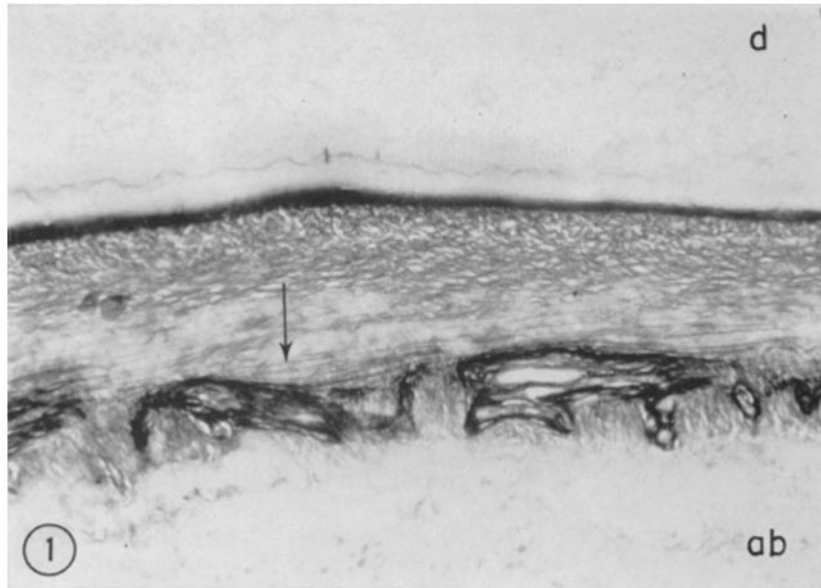
FIGURE 1   Periodontal membrane of the rat incisor stained by the oxidation–aldehyde fuchsin–Halmi sequence. The oxytalan fibers are stained darkly (arrow). The cementum also takes up the stain. The dentine (d) is in the upper part of the figure, and the alveolar bone (ab) in the lower. × 100.
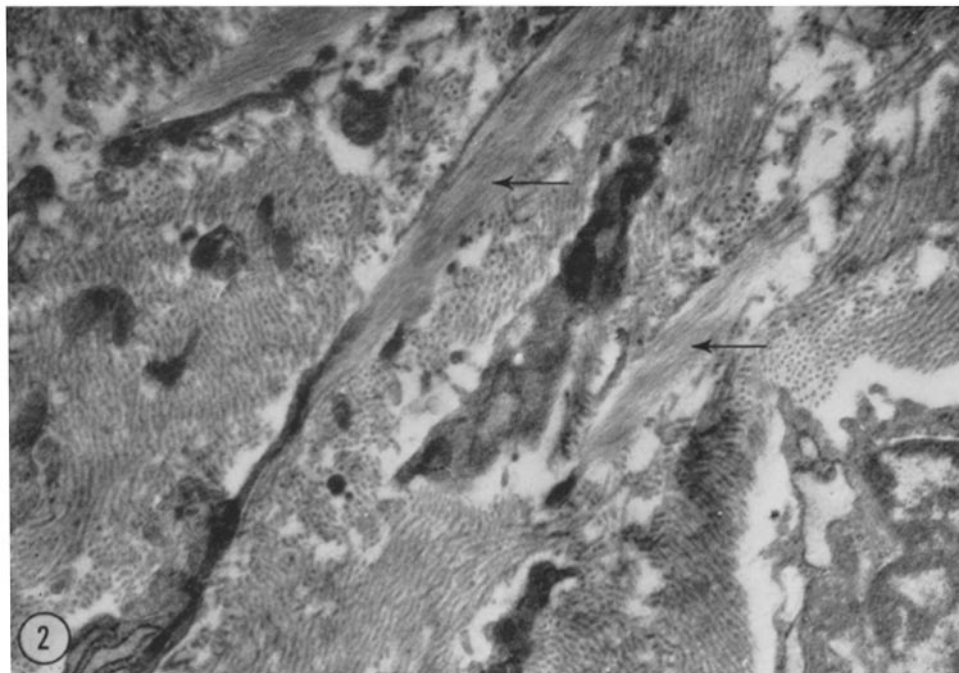

FIGURE 2   Electron micrograph showing three oxytalan fibers (arrows). × 12,790.
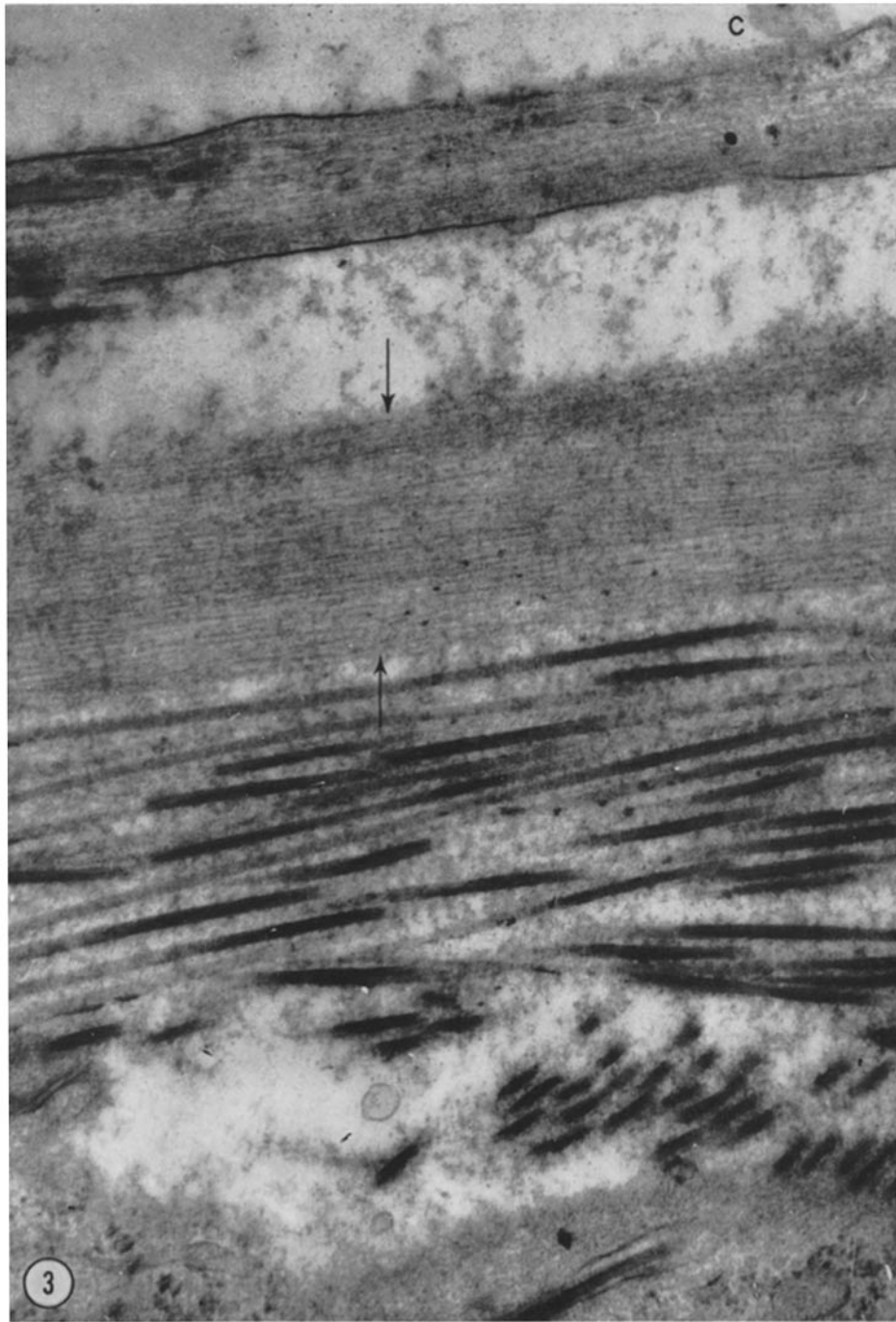
FIGURE 3 Higher magnification of the oxytalan fiber shown in the center between arrows. At the upper part of the figure (c) a cell process containing longitudinally arranged filaments is shown. × 35,500.

maining tooth was then sawn in an apico-occlusal direction through the pulp, to a point just short of the gingival attachment. These pieces of tooth were then postfixed in Palade's 1.0% buffered osmium tetroxide for 2 hr in the cold and then for 2 hr at room temperature. Dehydration was carried out through graded alcohols and the tissues were embedded in one of the epoxy resins, of which Maraglas gave the most uniform infiltration. After exposing the tooth in the polymerised resin, decalcification of the specimen was effected within the block with 0.1 N hydrochloric acid in 4 hr. Sections were cut on a Porter-Blum microtome, doubly stained with phosphotungstic acid and lead citrate, and examined in an RCA EMU 2B microscope.

Light Microscopy

Sections of rat teeth, fixed in formol-calcium and decalcified with formic acid, were stained by the persulfate aldehyde fuchsin technique of Fullmer and Lillie (1958) and Rannie (1963). With 1.0-μ thick epoxy resin–embedded sections it was not possible to stain specifically the oxytalan fiber, although the oxidation step resulted in some increased general staining of the sections by the aldehyde fuchsin.

OBSERVATIONS

The morphology of the oxytalan fibers in the periodontal membrane of the rat incisor is shown at the light microscope level in Fig. 1. No elastic fibers, as shown by conventionally used stains (aldehyde fuchsin, orcein, resorcin fuchsin, orcinol-new fuchsin and Verhoeff's) are present in this site.

Under the electron microscope, the only hitherto undescribed connective tissue element consists of bundles of filaments gathered together, without any apparent definite structural component linking them together. There is apparently ground substance within the bundles, but it seems to be present in much the same quantity as in the bundles of collagen and in the interfibrous spaces. These bundles of filaments are shown in Figs. 2 and 3 (arrows). The filaments are approximately 150 to 160 A in diameter, and the spaces between the filaments are of about the same diameter.

The filaments do not show any regular periodicity, and are of indefinite length.

The fibers were most frequently seen in the intermediate zone and region adjacent to the lamina dura of the periodontal membrane. In the zones adjacent to the cementum, the fibers were were seen only occasionally.

DISCUSSION

The electron microscope observations are in agreement with the conclusions drawn from the histochemical evidence regarding the nature of the oxytalan fiber. At the ultrastructural level there is a morphological similarity between the filaments of the oxytalan fiber and those of elastic tissue, although there is a size discrepancy. However, Ayer (1964), in reviewing the morphology of the elastin filaments, has concluded that the reported variations in diameter are due to differing degrees of aggregation of smaller structural elements. The chemical and physical properties of the oxytalan fiber have not been investigated completely, and it is not known whether the structure is static or whether it changes with alterations in physiological or pathological conditions.

Received for publication 6 July 1965.

REFERENCES

AYER, J. P., 1964, *Internat. Rev. Connective Tissue Research*, **2**, 33.

FISHER, A. K., and FULLMER, H. M., 1962, *Oral Surg., Oral Med., Oral Path.*, **15**, 246.

FULLMER, H. M., 1959, *J. Dent. Research*, **38**, 510.

FULLMER, H. M., 1960 a, *A.M.A. Arch. Path.*, **70**, 59.

FULLMER, H. M., 1960 b, *J. Histochem. and Cytochem.*, **8**, 290.

FULLMER, H. M., and LILLIE, R. D., 1958, *J. Histochem. and Cytochem.*, **6**, 425.

KOHL, J., and ZANDER, H. A., 1962, *Paradontologie*, **1**, 23.

RANNIE, I., 1963, *Trans. European Orthodontic Soc.*, **39**, 127.