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Oxytalan fibres in the rat pineal gland

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INTRODUCTION

Oxytalan fibres were first described by Fullmer & Lillie (1958). These fibres have been included in the 'elastic system' which is composed of elastic, oxytalan, and elaunin fibres (Cotta-Pereira, Guerra Rodrigo & David Ferreira, 1978). According to Fullmer (1960), oxytalan fibres are characterised by: (*a*) not staining by the classical elastin staining methods; (*b*) staining by these methods after a pre-oxidation with peracetic acid; (*c*) being found in normal adult tissues. Ultrastructural studies show that oxytalan fibres are composed of bundles of fibrils of 10–15 nm diameter. These fibrils, which present no transverse striation, are similar to the fibrillar component of elastic fibres (Carmichael & Fullmer, 1966; Cotta-Pereira, Guerra Rodrigo & David Ferreira, 1976, 1978; Soames & Davies, 1978; Alexander, Clayton, Howes & Garner, 1981).

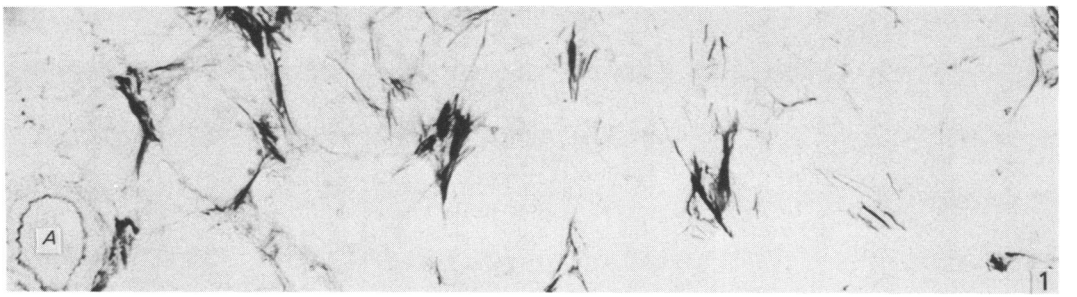
Oxytalan fibres have been described in different sites, such as the periodontal ligament (Sims, 1975; Soames & Davies, 1978), dermis (Cotta-Pereira *et al.* 1976), and cornea (Alexander *et al.* 1981). Until the present they had not been described in the pineal gland, but are now shown to occur in the rat pineal gland, using light and electron microscopy.

MATERIAL AND METHODS

Sixty eight male and female albino rats (Wistar), of ages between 1 day and 29 months, have been used for this study. Half the pineal glands were fixed in 10% neutral formalin and Bouin's fluid and embedded in paraffin. Serial sections 7 μm thick were obtained for light microscopy. These sections were stained with Gomori's aldehyde–fuchsin, orcein, and Verhoeff's iron haematoxylin, with and without pre-oxidation with peracetic acid (Fullmer & Lillie, 1958; Fullmer, 1960). For electron microscopy, the other half of the pineal glands was fixed by immersion in phosphate buffered 3% glutaraldehyde, post-fixed in 1% phosphate-buffered osmium tetroxide, and embedded in Vestopal or Epon. The sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 201 microscope.

RESULTS

Using the light microscope, oxytalan fibres were first identified in pineal glands of 3 weeks old albino rats, where they were located in the larger connective tissue septa. In animals 30–45 days of age, these fibres were found in most of the connective tissue spaces, forming small thin bundles in some cases. During the following months a slow and gradual increase in the amount of fibres was observed. In animals 10–12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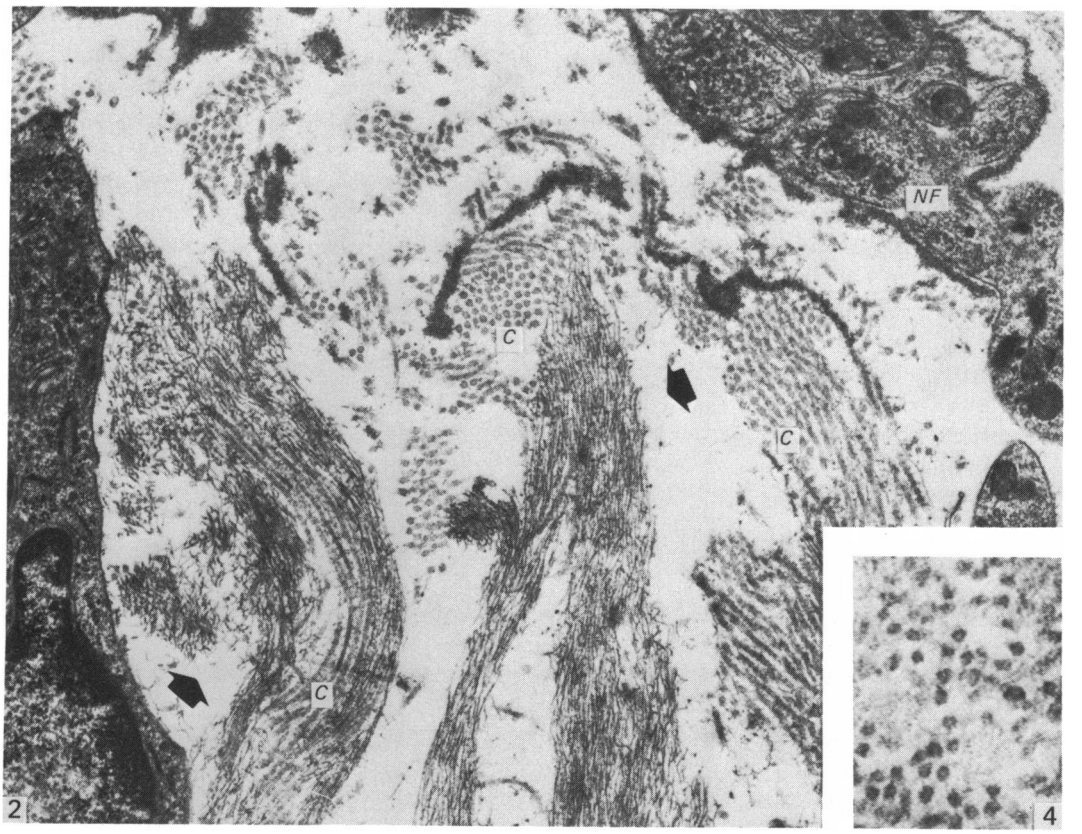
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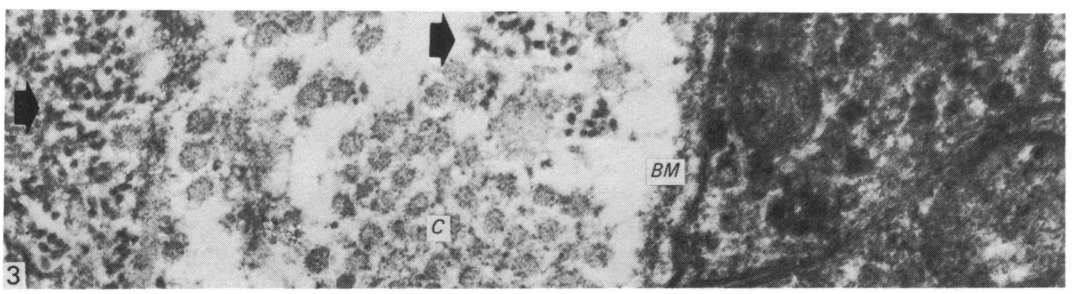
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months of age this increase was much more marked and large bundles of fibres were found, mostly located in the periphery near the pineal capsule (Fig. 1). The largest amount of fibres was found near the distal end of the pineal gland.

The oxytalan fibres of the pineal gland were identified by their staining characteristics; they stained with aldehyde–fuchsin and orcein (but not with the Verhoeff method) only after a pre-oxidation with peracetic acid (Fig. 1).

By electron microscopy, the connective tissue spaces of the albino rat pineal gland showed thin fibrils (12–15 nm) lacking a transverse striation (Fig. 2) and either forming bundles or interspersed among collagen fibres (Figs. 2, 3). The fibrils found inside the bundles were not arranged in a regular fashion (Fig. 2) and sometimes occurred in large disordered masses. At high magnifications, transverse sections of the fibrils displayed an apparently empty central space (Fig. 4). No definite relation was observed between the fibrils and any of the other components of the connective tissue spaces, including the parenchymal and vascular basement membranes (Figs. 2, 3).

All other findings with the electron microscope confirmed the light microscopy, although, with the former method, a few fibrils could be recognised as early as the second week after birth.

DISCUSSION

These results show the existence of oxytalan fibres in the connective tissue spaces of the albino rat pineal gland, fibres which fulfil the staining requirements established by Fullmer (1960). The techniques used for elastic fibres (without previous oxidation of the sections in peracetic acid) show practically none of these fibres in connective tissue spaces of the rat pineal gland, even in specimens of advanced age (29 months). This discounts the possibility that the fibres described be considered pre-elastic fibres. The study of connective tissue spaces, using electron microscopy, shows fibrils whose ultrastructural appearance agrees with the previous descriptions for oxytalan fibres (Carmichael & Fullmer, 1966; Cotta-Pereira *et al.* 1976, 1978; Soames & Davies, 1978). Also, the tubular structure now found in pineal oxytalan fibrils has previously been described for this type of fibre ('fibrotubules' of Cotta-Pereira *et al.* 1976).

The number of pineal oxytalan fibres increases with age and considerable amounts may thus be found in older animals. Johnson (1980) describes 'filamentous masses' in the pineal connective tissue spaces of old rats. These masses may correspond to oxytalan fibres. Krstic (1979) also describes 'amorphous granular masses' in the pineal connective tissue spaces of the rat, using scanning electron microscopy on criofractured material. The transmission electron micrograph which accompanies

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Fig. 1. Male rat, 18 months old. Aldehyde–fuchsin technique for oxytalan fibres, without nuclear stain. Some connective septa show bundles of fibres with a positive reaction. *A*, artery. × 350.

Fig. 2. Male rat, 12 months old. Connective tissue space showing thin fibrils (arrowheads) forming small bundles interspersed among the collagen fibres (*C*). *NF*, nerve fibres. × 16500.

Fig. 3. Female rat, 10 months old. Transverse section of collagen fibres and oxytalan fibres (arrowheads) of lesser diameter. *BM*, basement membrane of pineal parenchyma. × 70000.

Fig. 4. Male rat, 6 months old. Transverse section of oxytalan fibres. There is a lesser central density, which suggests a tubular structure. × 130000.

his description shows thin fibrils which could also correspond to oxytalan fibres. Neither Johnson (1980) nor Krstic (1979) identifies the fibrils as oxytalan fibres.

SUMMARY

The present study shows the existence of oxytalan fibres in the connective tissue spaces of the rat pineal gland. The identification of these fibres with light microscopy is based on their ability to stain with aldehyde–fuchsin and orcein after oxidation with peracetic acid. Using the electron microscope, oxytalan fibres appear as bundles of fibrils of 12–15 nm without transverse striation. Oxytalan fibres increase with age, being most abundant in the old rat.

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