J. Anat. (1995) **187**, pp. 353–359, with 3 figures Printed in Great Britain

Characterisation of the neurosensory elements of the feline cranial cruciate ligament

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(*Accepted 7 March 1995*)

ABSTRACT

The identification and distribution of mechanoreceptors in the cranial cruciate ligament of the cat (analogous to the anterior cruciate ligament in other species) was studied histologically using a modified celloidin embedding technique to achieve serial sectioning of bone–ligament–bone preparations with gold chloride staining. We identified distinctive large elongated structures situated between the collagen bundles of the ligament (resembling endings described as Freeman and Wyke type III; also termed Golgi tendon receptors). These endings were found near the middle of the ligament well away from the bone–ligament junction. Axons seen entering only one end of each type III ending helped to confirm its neural basis. While we saw structures resembling types I and II endings (i.e. Ruffini and pacinian endings, respectively) in individual sections, serial sections failed to reveal convincing evidence of their existence. Such structures almost always appeared to be vascular in nature on adjacent sections, with vessels entering and exiting. We conclude that serial sections are critical to interpreting the presence or absence of mechanoreceptors.

Key words: Cat; knee; Golgi tendon organs.

INTRODUCTION

Ligaments are generally viewed as passive structures used for support, stabilisation, and restriction of motion. Conversely, torn ligaments are usually believed to lead exclusively to structural instability. Treatment is based almost entirely on this assumption, and surgical repair and reconstruction focus purely on structural restoration. Substantial evidence suggests, however, that ligaments serve another major role: as sources of signals for reflex functions in the neuromuscular system. Should this be true, and if symptoms and a reduction in function occur because of a loss of this activity as a consequence of the injury, then current treatments do little or nothing else to help restore this lost function (Brand, 1989).

One of the most convincing pieces of evidence for a neurosensory function is the presence of ligament mechanoreceptors, first reported in 1860 by Krause. However, many authors disagree on the composition of the neurosensory elements of the anterior cruciate ligament (the most commonly injured ligament causing dysfunction). For example, Kennedy et al. (1974, 1982) and Goertzen et al. (1992) demonstrated only Golgi tendon organs and free nerve endings within the cruciate ligaments while Freeman & Wyke (1967) demonstrated 4 types of mechanoreceptors including Ruffini endings and pacinian corpuscles in addition to Golgi tendon organs and free nerve endings.

The seemingly contradictory conclusions raise questions regarding the identification and interpretation of what is and what is not a mechanoreceptor, a process inevitably somewhat subjective. DeAvila et al. (1989), studying multiple sections, commented that 'nerve endings identified in the present study did not resemble putative endings in photomicrographs of portions of anterior cruciate ligaments from human beings in previous studies'. With regard to previous interpretations of mechanoreceptors, they stated 'indeed the morphological features of those "endings" were either not discernible in the photomicrographs or closely resembled structures unequivocally identified as blood vessels in the present study'. Dykes (1984) suggested that there are so many variations in the

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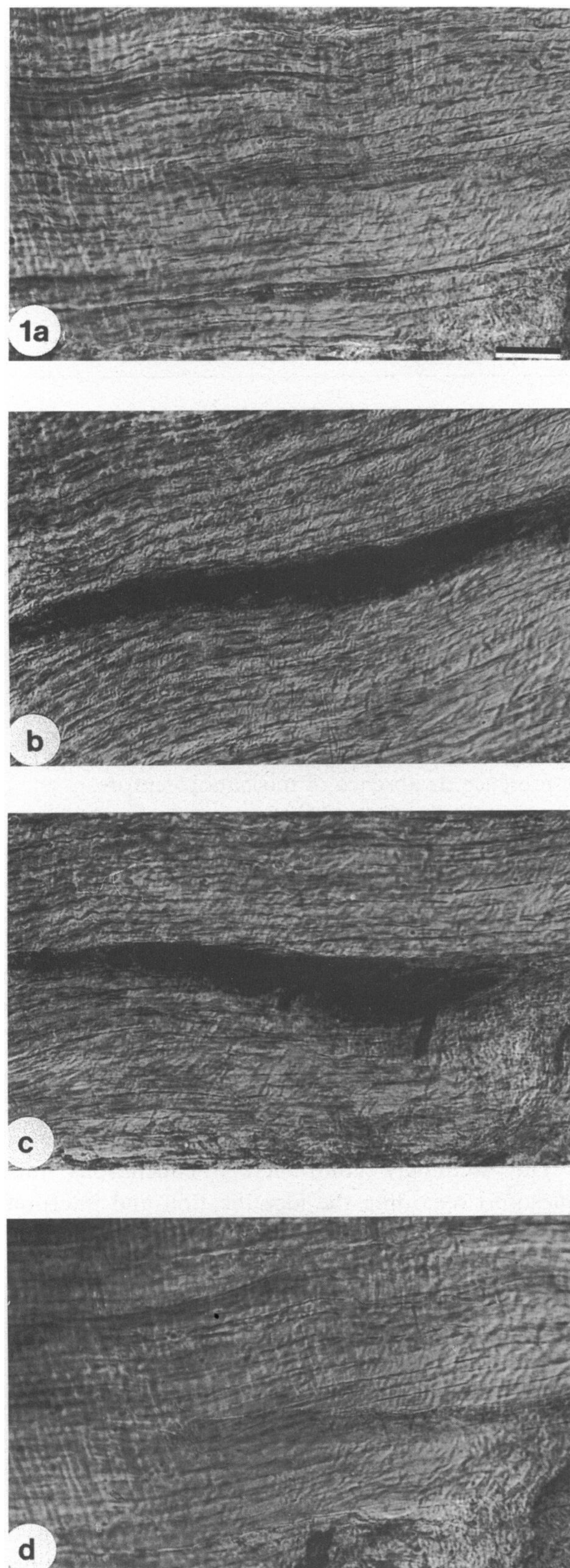

Fig. 1. Serial sections (*a–d*) of a type III receptor. The structure is aligned with the long axis of the ligament within the collagen bundles. An axon is seen entering and the structure exhibits a spiralled appearance and a fusiform shape. The structure is seen in only 2 serial sections (*b*, *c*). Bar, 100 μm.

morphological characteristics of sensory receptors that they represent a continuum rather than distinct classes.

In addition to the problems of identification and interpretation of ligament mechanoreceptors, there is a question as to whether methods of preparation destroy the receptors they are intended to find. Several investigators have described the majority of receptors near the ligament ends (Freeman & Wyke, 1967; Kennedy et al. 1982; Schultz et al. 1984; Schutte et al. 1987; Gomez-Barrena et al. 1992), yet most preparations cut the ligaments away from bone, perhaps destroying or eliminating some receptors. In fact, Gomez-Barrena et al. (1992) stated 'the number of these nerve endings depends on the cutting plane and how the ligament was obtained (the number decreases if the insertions are sectioned without bone)'. We therefore developed a technique to allow serial sectioning of bone–ligament–bone preparations. The purpose was to ascertain whether or not what appeared to be a receptor on one section would appear to be a receptor on subsequent sections. Analysing the intact ligament with its insertions would avoid any potential destruction of receptors near the ends.

METHODS

The method is modified from that used by Zimny (1985) and DeAvila et al. (1989), differing in processing times, washing, embedding technique, and sectioning technique. Ten cranial cruciate ligaments including femoral and tibial insertions were excised from the hind limbs of 5–10 mature cats killed in another study by potassium chloride injection after alpha-chlorolose anaesthesia; both studies had the approval of our institutional animal welfare committee. Approximately $2 \times 4 \times 6$ mm of bone was removed with the insertion of the ligament to ensure a true bone–ligament–bone specimen.

The tissue was submerged in 1 part 88% formic acid to 3 parts filtered lemon juice (Borden's ReaLemon) and placed in the dark for up to 6 h or until the tissue was transparent. The formic acid solution was decanted and the tissue blotted. A 1% solution of aqueous gold chloride was added and the tissue returned to the dark for 1 h or until the tissue turned a uniform golden yellow. The gold chloride solution was decanted and 25% formic acid was added to cover the tissue. The tissue was kept in the dark for 24 h at 4 °C to ensure slow oxidation of the gold chloride and decalcification of the bone. The 25% formic acid was decanted and the tissue washed

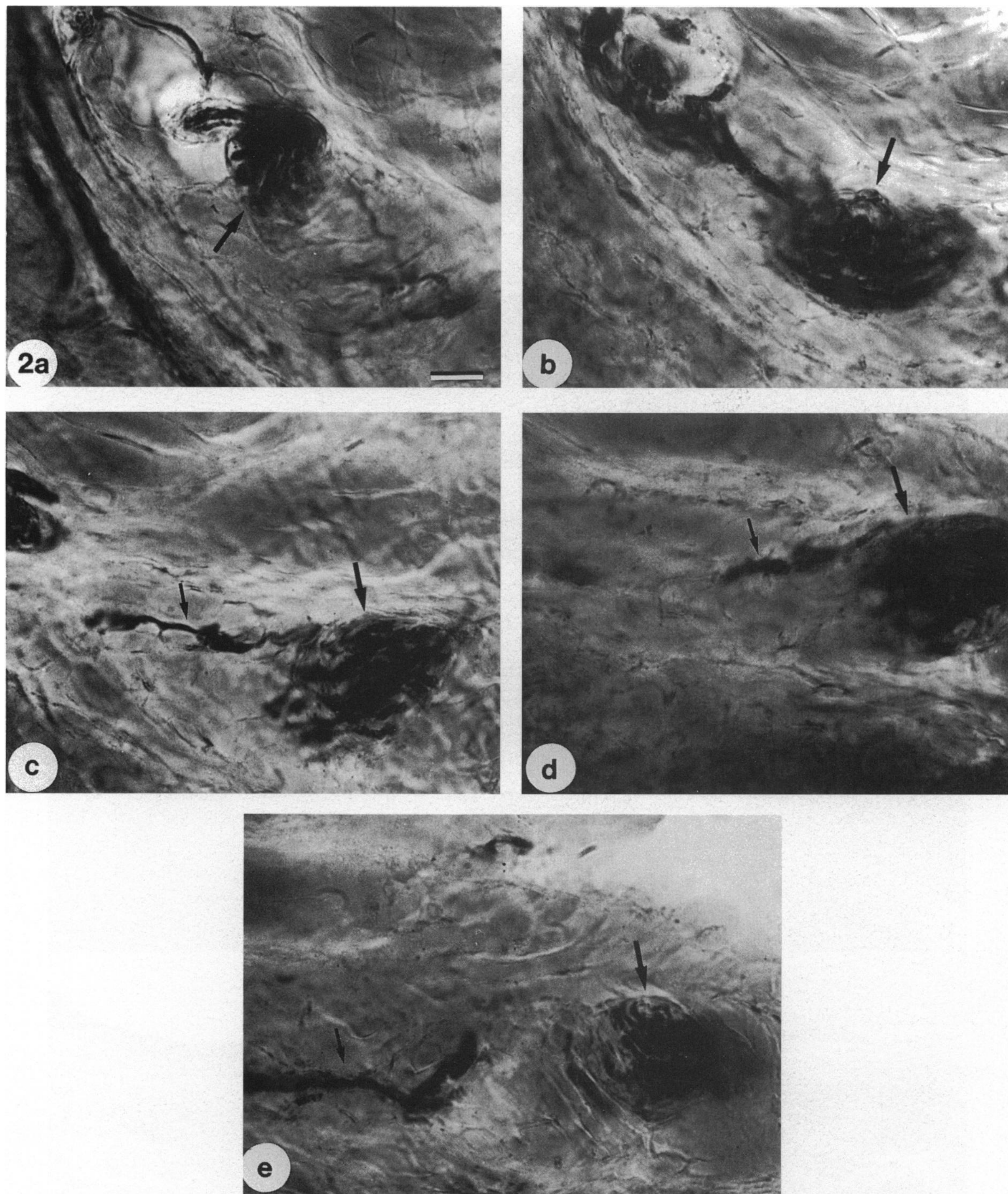

Fig. 2. Serial sections (*a–e*) demonstrate a structure having the appearance of a Ruffini ending in a single section (*c*). The linear structures in *c*, *d*, and *e* marked with small arrows appeared to arise from small blood vessels on sections beyond those shown; we interpreted the marked structures as blood supply to a peripheral nerve. The larger black structures (approximately 40 × 60 μm in sections *a–e*, marked with large arrows) contained distinct bundles consistent with a peripheral nerve; this structure seen in 10 serial sections (each 70 μm in thickness) is not consistent in size or configuration with a solitary neural ending. Bar, 20 μm.

in 3 changes of 95% alcohol under vacuum, 3 changes of absolute alcohol under vacuum and 3 changes of ether: alcohol under vacuum. This step removed the formic acid from the soft ligamentous tissue and hardened the tissue. The tissue was placed in 2% celloidin ethyl ether: alcohol solution under vacuum for 2–3 d. Dry desiccant was placed in the vacuum with the tissue container to ensure the celloidin solution did not hydrate. The tissue was next transferred to a 4% celloidin solution under vacuum for 1–2 d, then to a sealed container of 8% celloidin solution and left for at least 14 d to ensure complete infiltra-

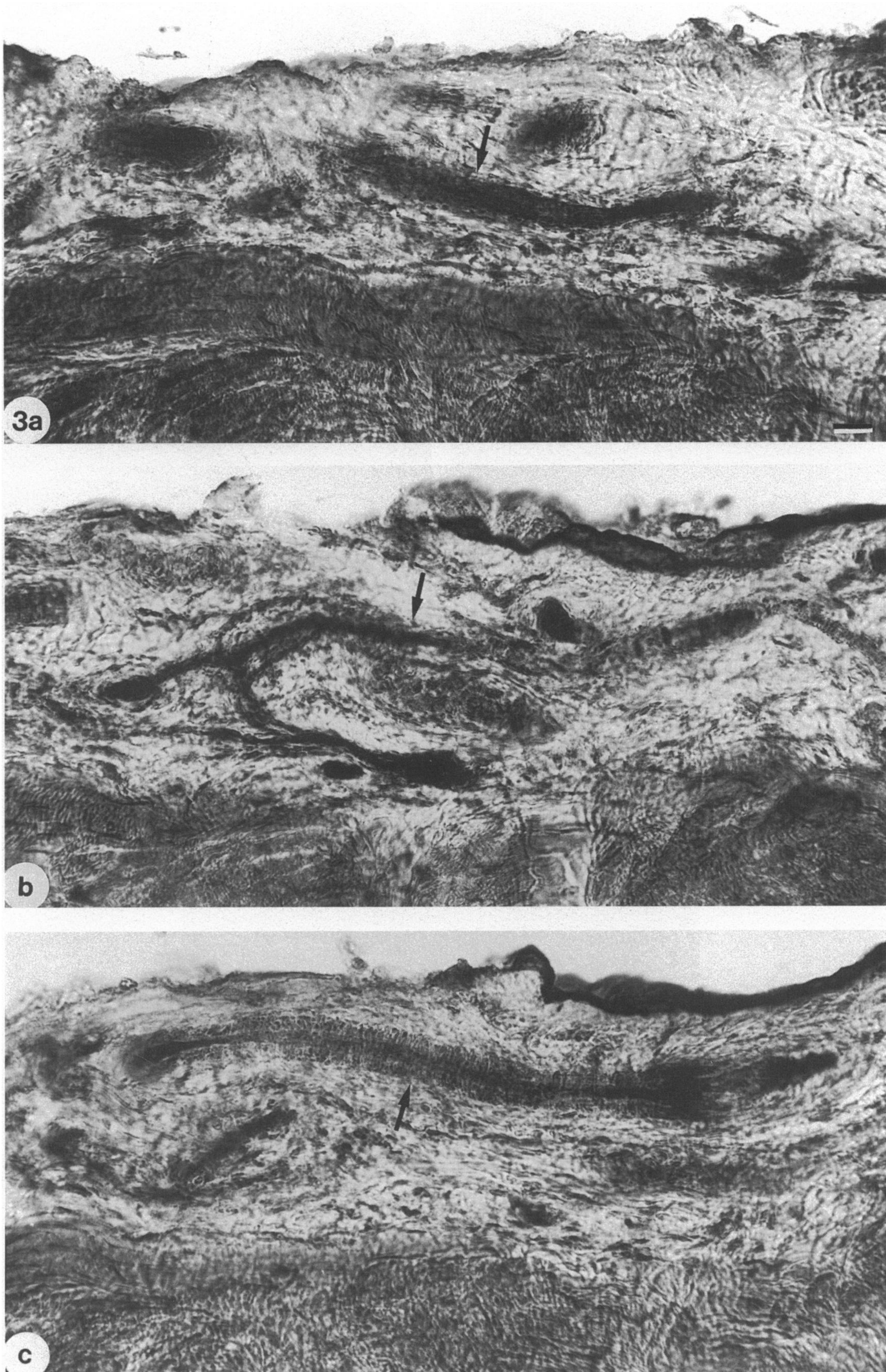

Fig. 3*a*–*c*. For legend see opposite.

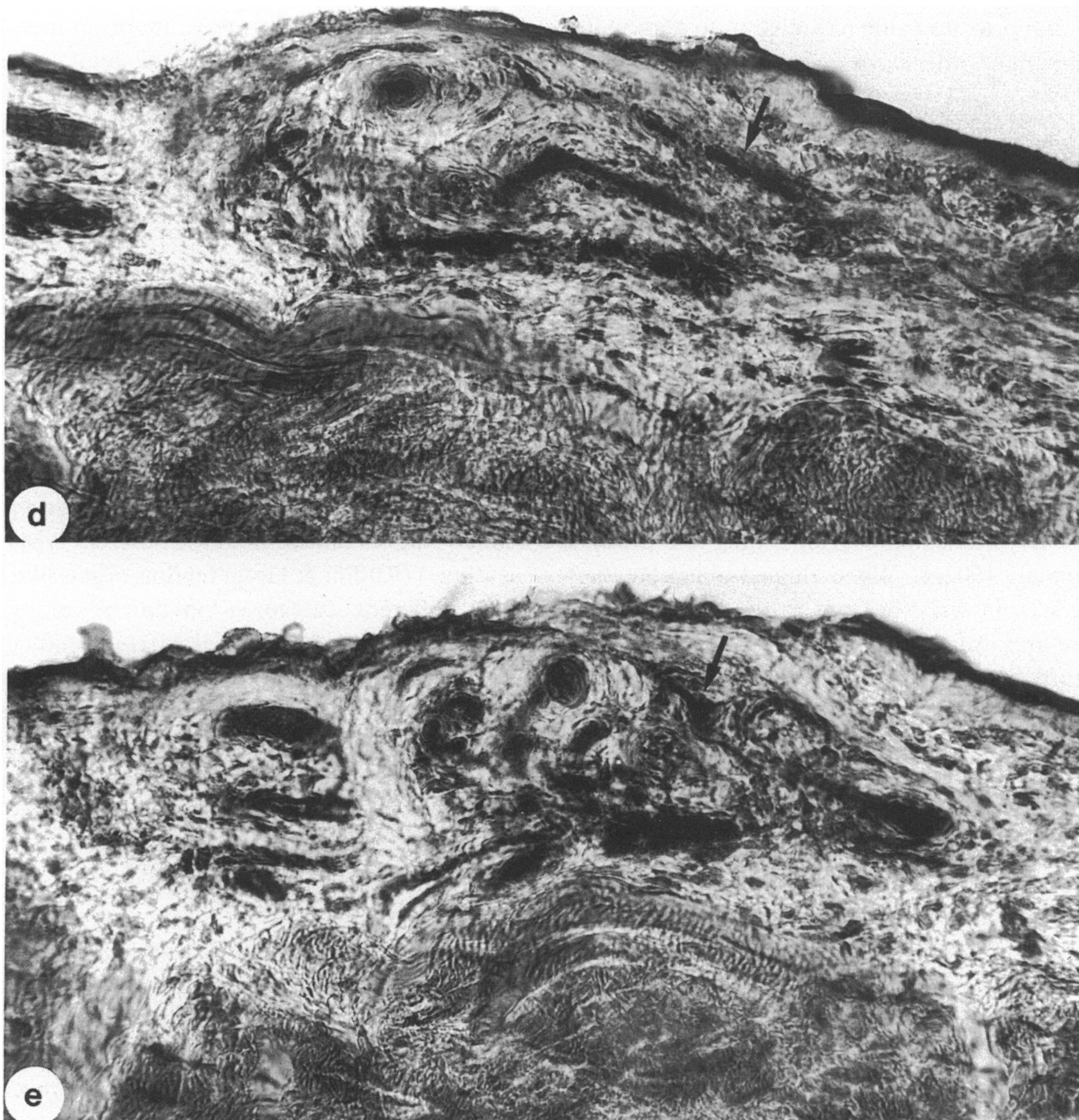

Fig. 3. In (*c*) a structure is visible similar to those illustrated in Freeman & Wyke (1967), Zimny (1988) and Sjölander et al. (1989) (and termed pacinian corpuscle). When viewed in serial sections (*a–e*) the structure is seen to be part of a vascular network of interconnecting vessels. Bar, 25 μm.

tion. The tissue remained submerged in celloidin solution. The tissue container cover was replaced with Parafilm to facilitate very slow drying of the 8% celloidin solution. When a finger no longer left a print in the celloidin overlying the tissue, the specimen was cut from the dish leaving approximately 0.5–1 cm of embedding material around the tissue. A paper mould approximately 10 cm high was formed around a wooden mounting block. A 12% celloidin solution was placed in the bottom of the mould to a depth of approximately 2 cm and allowed to form a film. The tissue was then placed on the 12% solution already in the mould being careful to orient the tissue within the mould at this time. The tissue was oriented with the long axis of the ligament parallel to the surface of the embedding solution. The wide insertion of the tibial end of the ligament could be identified to ensure proper orientation of the tissue. 12% celloidin solution was then added to cover the tissue. This mould was placed in a closed container along with chloroform vapour to harden.

When hardened, the paper was removed and the block was cut on a horizontal sliding microtome at approximately 70 μm thickness. Because celloidin is a transparent embedding medium, the wide tibial insertion could be identified and proper orientation maintained. Prior to sectioning, the block could be adjusted within the microtome vice to ensure sectioning of the tissue block parallel to the long axis of the ligament. The block was kept moist with 70%

alcohol while cutting. Cut sections were stored in 70% alcohol prior to being mounted onto glass slides and coverslipped with a synthetic mounting medium.

RESULTS

We observed 1 to 3 stained structures within the deep collagenous substance of each feline cranial cruciate ligament; the structures resembled those described by Freeman & Wyke (1987) as a type III ending (Fig. 1). These structures exhibited a characteristic fusiform shape approximately 100 × 600 μm and were aligned with the long axis of the ligament. Axons entering the structures branched and assumed a spiralling appearance. These structures, when aligned parallel to the plane of the section were almost always seen in at least 2 serial sections thus corresponding to a width of approximately 100 μm. When aligned obliquely, they could be seen in serial sections in the corresponding respective areas of the ligament.

Structures resembling those illustrated and interpreted by DeAvila et al. (1989) and Sjölander et al. (1989) as type I receptors (Ruffini endings) were seen on individual sections (Fig. 2 *c*). When analysed serially these structures proved to be part of either peripheral nerves or capillaries (Fig. 2). Capillaries sometimes entered these structures and exhibited a 'lattice work' pattern of cell junctioning characteristic of vascular connective tissue.

Structures resembling type II receptors (pacinian corpuscles) were also identified on individual sections (Fig. 3*c*) (Zimny, 1988; Sjölander et al. 1989). When analysed serially (Fig. 3) these structures possessed 8–10 layers of 'capsular' connective tissue characteristic of arterioles. On subsequent serial sections these structures branched and interconnected with other structures having the histological appearance of blood vessels (multiple layers of smooth muscle cells with a clearly discernible continuous lumen).

DISCUSSION

On individual sections, we observed structures similar to all 3 types of mechanoreceptors previously reported by various authors. Polacek (1966) described 'spray' and 'rosary-like' endings found primarily in the joint capsule and superficial portions of the ligaments of numerous species including felines. Freeman & Wyke (1967), while noting 4 types of mechanoreceptors, concluded that the characteristic end organ in feline intrinsic (cruciate) ligaments was the type III (Golgi tendon organ) ending. Zimny (1988), also in a study of cat cranial cruciate ligaments, described 4 types of mechanoreceptors but noted pacinian corpuscles and Golgi tendon organs were more numerous than Ruffini endings. In a study of human ligaments, Kennedy et al. (1974, 1982) described only Golgi tendon organs and free nerve endings within the anterior cruciate ligament. Similarly Goertzen et al. (1992) noted only Golgi tendon organs and free nerve endings in the dog anterior cruciate ligaments. Badalamente et al. (1984) described type I (Ruffini) endings in the rat and human but denied the presence of type II endings within the anterior cruciate ligament. DeAvila et al. (1989) noted 2 types of 'non-paciniform' endings in the anterior cruciate ligament of the dog. Johansson et al. (1991) in a study of human anterior cruciate ligaments described all 4 Freeman and Wyke types of mechanoreceptors but stated 'the classification of the different types of spray endings' (Ruffini & Golgi tendon organ-like endings) into different categories might be more or less arbitrary'. Haus & Halata (1990) in a study of human ligaments noted Ruffini corpuscles and pacinian corpuscles but no Golgi tendon organs and, regarding interpretation, stated 'nerve corpuscles have been described but the structures shown needed to be interpreted with great imagination'. Thus there is considerable variability as to findings, perhaps in part owing to interpretive differences as suggested by these latter authors.

Another consideration for varying conclusions may be the variation in species between various histological studies. Polacek (1966) stated 'the encapsulated corpuscles, even the spray-like endings are subjected to profound species differences'. However, examination of our serial sections of feline cranial cruciate ligaments suggested that all of the structures resembling types I and II receptors were vascular. Only those structures resembling type III receptors proved to have the characteristics expected to nervous end-organs (i.e. an axon entering but not exiting, and a fusiform shape aligned within the collagen bundles of the ligament).

DeAvila et al. (1989) noted that structures appearing to be mechanoreceptors 'closely resemble structures unequivocally identified as blood vessels'. While they apparently analysed multiple sections in reaching this conclusion, they did not report results of serial sections. We concur, however, with their conclusions. Serial sectioning appears to be a useful and indeed perhaps a critical part of identifying ligament mechanoreceptors.

ACKNOWLEDGEMENTS

This work was supported in part by an Award for Excellence in Research administered through the Orthopaedic Research and Education Foundation from Bristol-Myers/Zimmer and NIH grant RO1AR40199. We would also like to thank Dr Jerry Maynard for valuable advice.

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