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Chick vincula: elastic structures with a check-rein mechanism

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INTRODUCTION

Vincula are small bands which connect flexor tendons to phalanges, usually different ones from those into which the tendons are inserted. According to Hollinshead (1965) their primary function is to carry a blood supply to tendons within tendon sheaths, but he also states that 'the short vincula, especially, may allow the tendons to have some direct action on phalanges proximal to their insertion'.

In man these bands are often mere slips of connective tissue. From injection studies Brockis (1953), Chaplin (1973), Edwards (1946) and Verdan (1972), have shown that they contain tiny vessels. However, in the chick, vincula are much larger and more complex structures than in men. They certainly contain vessels which supply the tendon within the synovial sheath, but these vessels make up only a very small portion of the total mass; most of the structure consists of collagen and elastic fibres.

The purpose of this paper is to describe the structure, and to discuss the possible functions, of the chick vincula associated with the flexor profundus tendons. Particular attention will be paid to the elastic fibres and to the relationships of the collagen fibres to them.

MATERIALS AND METHODS

Three month old White Leghorn chickens obtained from a local hatchery were humanely killed and the feet removed and skinned. The digits were excised and the synovial sheaths slit open. Some digits were placed in fixative without further ado, so that the vincula were fixed in their short or 'relaxed' state. Other digits were pinned to a piece of tongue depressor in such a way that the vincula were in their long or 'stretched' state, and fixation was carried through without removing the pins.

The specimens were fixed in 4% glutaraldehyde in 0·1 M sodium cacodylate buffer. They were rinsed with the same buffer, post-fixed in 1% osmium tetroxide in *s*-collidine buffer (Bennett & Luft, 1959), dehydrated through a series of alcohols and propylene oxide, and embedded in Epon (Luft, 1961).

Thick sections were stained with Richardson's methylene blue (Richardson, Jarrett & Fincke, 1960). Thin sections were stained with aqueous uranyl acetate and Millonig's lead (Millonig, 1961) and then examined with an AEI 801 microscope.

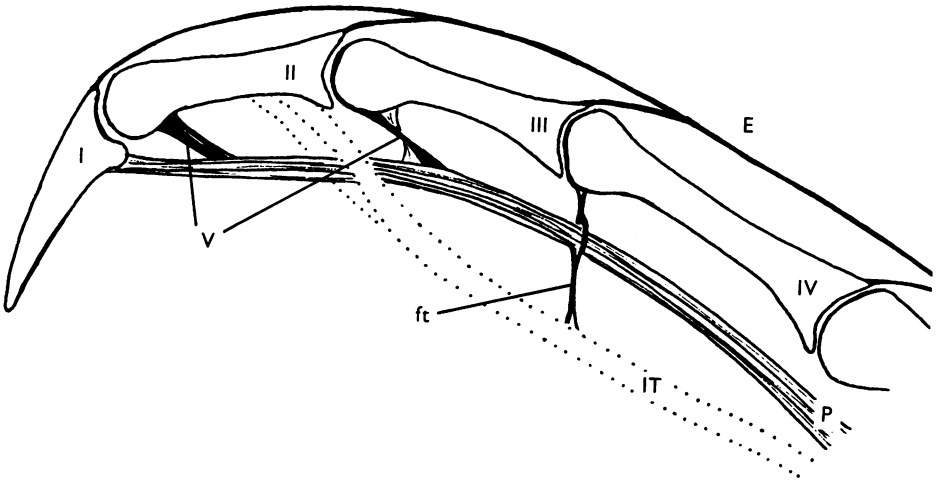


Fig. 1. Drawn from the third digit of the chick foot. The phalanges are numbered I–IV with the distal phalanx or claw being number I. The digit is shown in a somewhat flexed position with the tendons pulled away from the bones in order to show the positions of the vincula with their loose fibrous tissue attachments (V), the profundus tendon (P), the intermediate tendon (IT), and fibrous tissue (ft) attached to the intermediate tendon through which the profundus passes. The intermediate tendon becomes a part of the synovial sheath just as it splits. However, it inserts into the base of the second phalanx. For simplicity, the synovial sheath is not shown. In the 'at rest' position, the vincula are not stretched and are closely applied to the dorsum of the tendon. The extensor tendon (E) attachments are also illustrated.

RESULTS

Gross observations

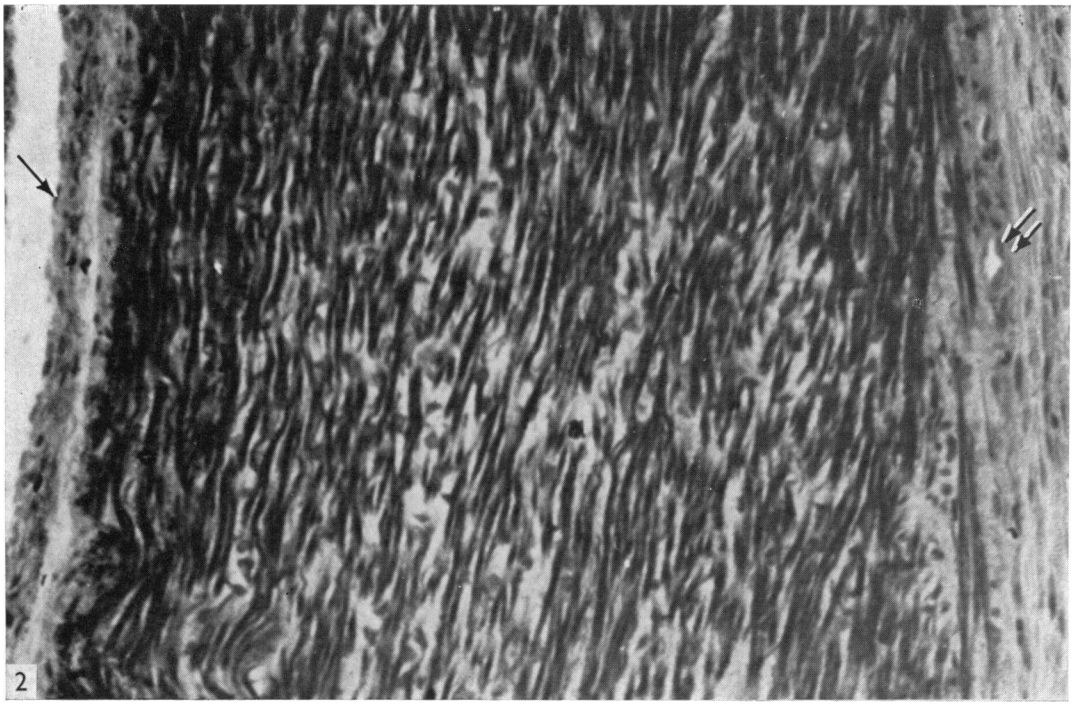
The vincula appeared as small yellow bands on the dorsal surface of the flexor profundus tendons within the synovial sheaths. There were two of them attached to each profundus tendon. They originated from the distal ends of the second and third phalanges (numbered from the tip of the digit) (Fig. 1), except in the case of the first digit which had a single vinculum arising from the second phalanx. (It should be remembered that in the chick each digit has a different number of phalanges; the first has two, the second has three, the third has four, and the fourth has five: Koch, 1973.) The short vinculum (*vinculum breve*) was inserted into the palmar surface of the distal end of the second phalanx. The long vinculum (*vinculum longum*) was inserted into the palmar surface of the distal end of the third phalanx.

Clearly the action of the flexor profundus muscle is primarily on the first (i.e. distal) phalanx, and the vinculum is stretched when the muscle contracts.

The vinculum-like structure that inserts into the intermediate tendon, and through which the profundus tendon passes (Fig. 1), will not be dealt with in this paper.

Light microscopy

The vincula were composed of densely packed elastic and collagen fibres, apart from a thin superficial layer which contained many cells and blood vessels but few if any elastic fibres (Fig. 2).



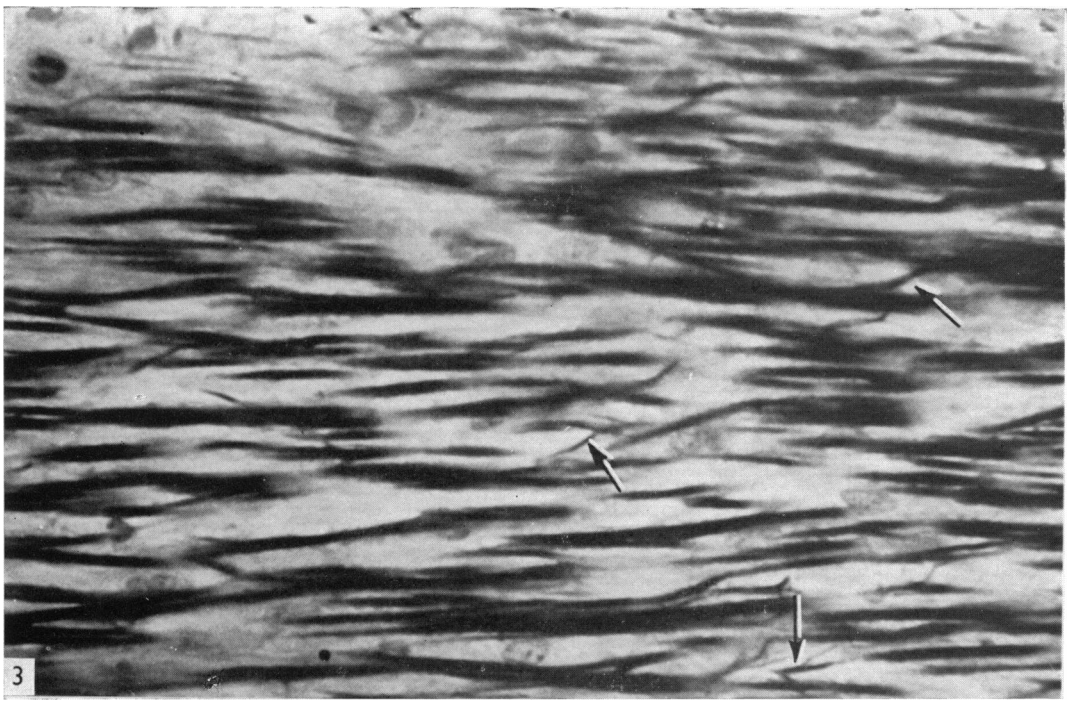


Fig. 2. Relaxed. The black lines are longitudinally sectioned elastic fibres. The superficial connective tissue contains a large vessel (double arrows). The outer synovial layer of the vinculum is also seen (arrow). × 320.

Fig. 3. Relaxed. The longitudinally sectioned fibres appear to branch to form either larger or smaller fibres (arrows). × 620.

With Richardson's stain the elastic fibres stained a dense blue but the collagen matrix and the cells were only very lightly stained. Cells were sparse and surrounded by groups of elastic fibres. Many of the elastic fibres branched (Fig. 3). No significant differences between stretched and relaxed vincula were observed with the light microscope.

Electron microscopy (general)

In *cross sections* the long axes of the superficial cells were at right angles to the long axis of the vinculum (Fig. 4). The outermost layer was made up of cells which were highly vacuolated, and they had a thin layer of fuzzy material on their outer side (Fig. 4, inset). The cells of the innermost layer were elongated and surrounded by a collagenous matrix with an occasional elastic fibre orientated at right angles to the cells. The intermediate layer was made up of cells that had some characteristics of both types of cell (Fig. 4). It was largely through this superficial cellular layer that blood vessels were carried to the tendon.

One of the most striking features of the cross sectioned vincula, whether relaxed or stretched, was the constant association of cytoplasmic processes with the elastic fibres (Fig. 5). In some cases the fibres were almost completely enveloped by cytoplasm (Greenlee, Ross & Hartman, 1966).

The cells in this main, elastic part of the vinculum had nuclei which were often highly lobulated in both stretched and relaxed states, but perhaps more so in the latter: their cytoplasm showed large numbers of ribosomes associated with an extensive endoplasmic reticulum, many microfibrils and some microtubules: there was also a highly organized Golgi apparatus (Fig. 6). The cells were, to all appearances, 'fibroblasts'.

The elastic fibres exhibited very few microfibrils, either internally or externally (Fig. 7). However, this may be a consequence of their size and age (Greenlee *et al.* 1966; Fahrenbach, Sandberg & Cleary, 1966; Haust *et al.* 1965; Albert, 1972), for the vincula examined were from chickens which were almost fully grown.

In *cross sections*, large elastic fibres could be seen branching into smaller fibres and smaller fibres coalescing into larger ones.

In *longitudinal sections* it could be seen clearly that the elastic fibres formed a branching network (Fig. 8), probably three-dimensional: this appearance was not just a consequence of accidental tangential sectioning (see Fig. 8, inset).

Electron microscopy (relaxed versus stretched vincula)

Relaxed: In cross sections some of the collagen fibres appeared to loop around the elastic fibres. Other collagen fibres were cut tangentially and transversely.

The longitudinally sectioned, relaxed vinculum exhibited a random collagen network. The elastic fibres appeared to run parallel for only short distances, either because of tangential sectioning, or because the fibres were wavy. Fig. 9 illustrates

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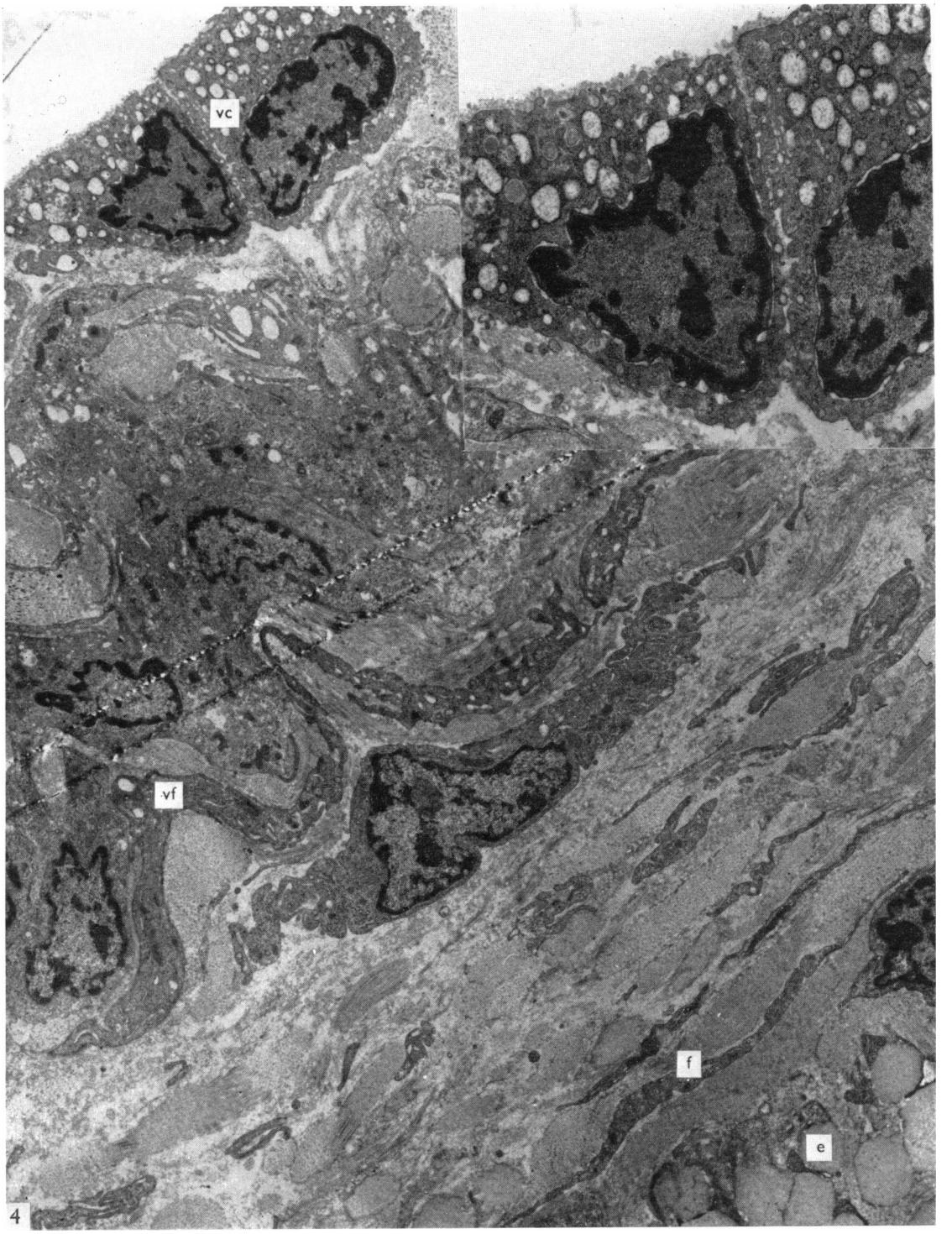
Fig. 4. Stretched. The cellular layers of the vinculum show the highly vacuolated cells (vc) of the outer layer, the inner layer of elongated cells (f), and the intermediate layers with cells (vf), characteristic of both layers. The cross sectioned elastic fibres (e) of the vinculum are also seen. × 6800. Insert. Stretched. This is a higher magnification of cells seen in Fig. 4 to show the fuzzy layer on the synovial layer of cells. × 13400.



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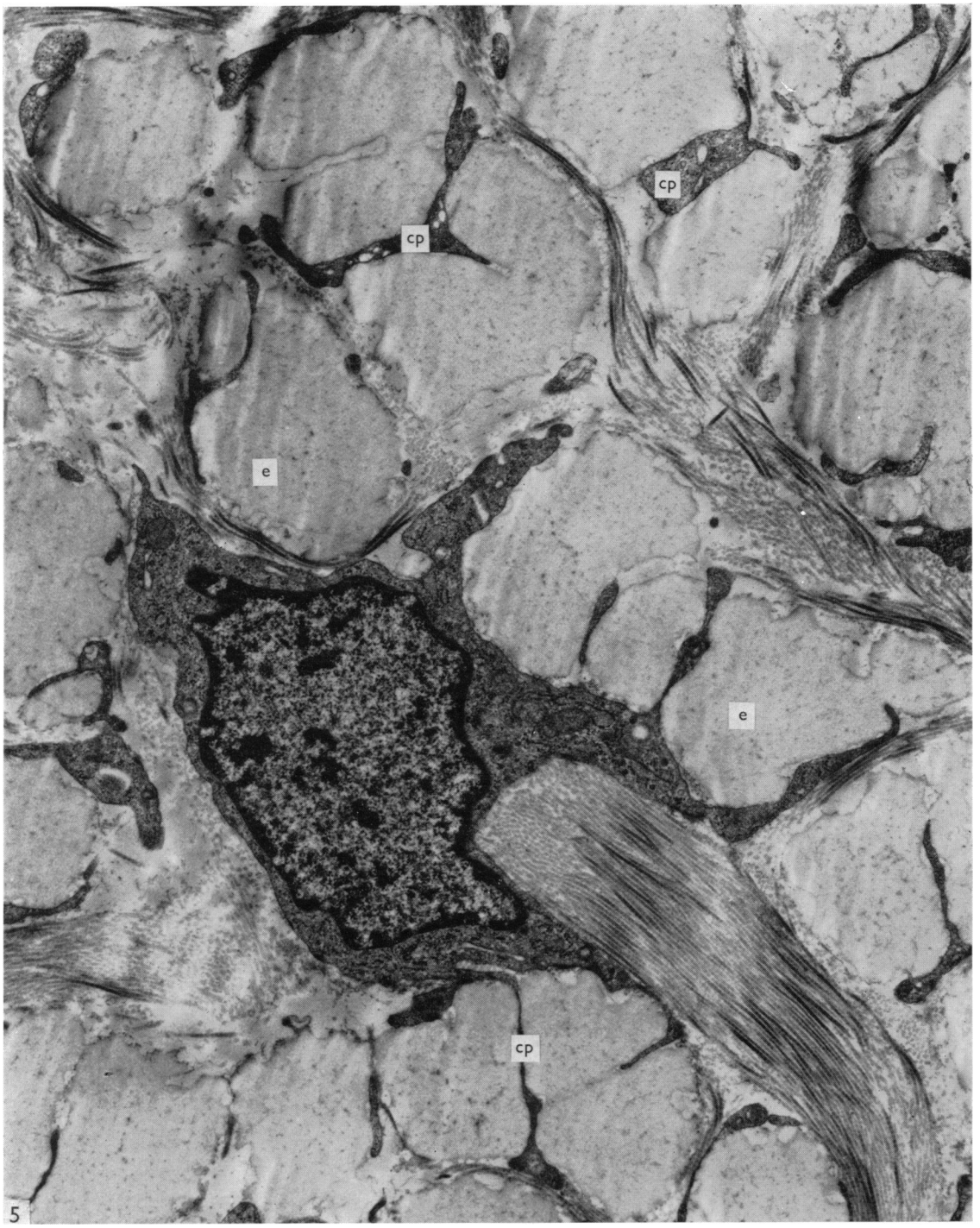

Fig. 5. Relaxed. Cross sectioned elastic fibres (e) are very closely associated with cytoplasmic processes (cp). ×11400.

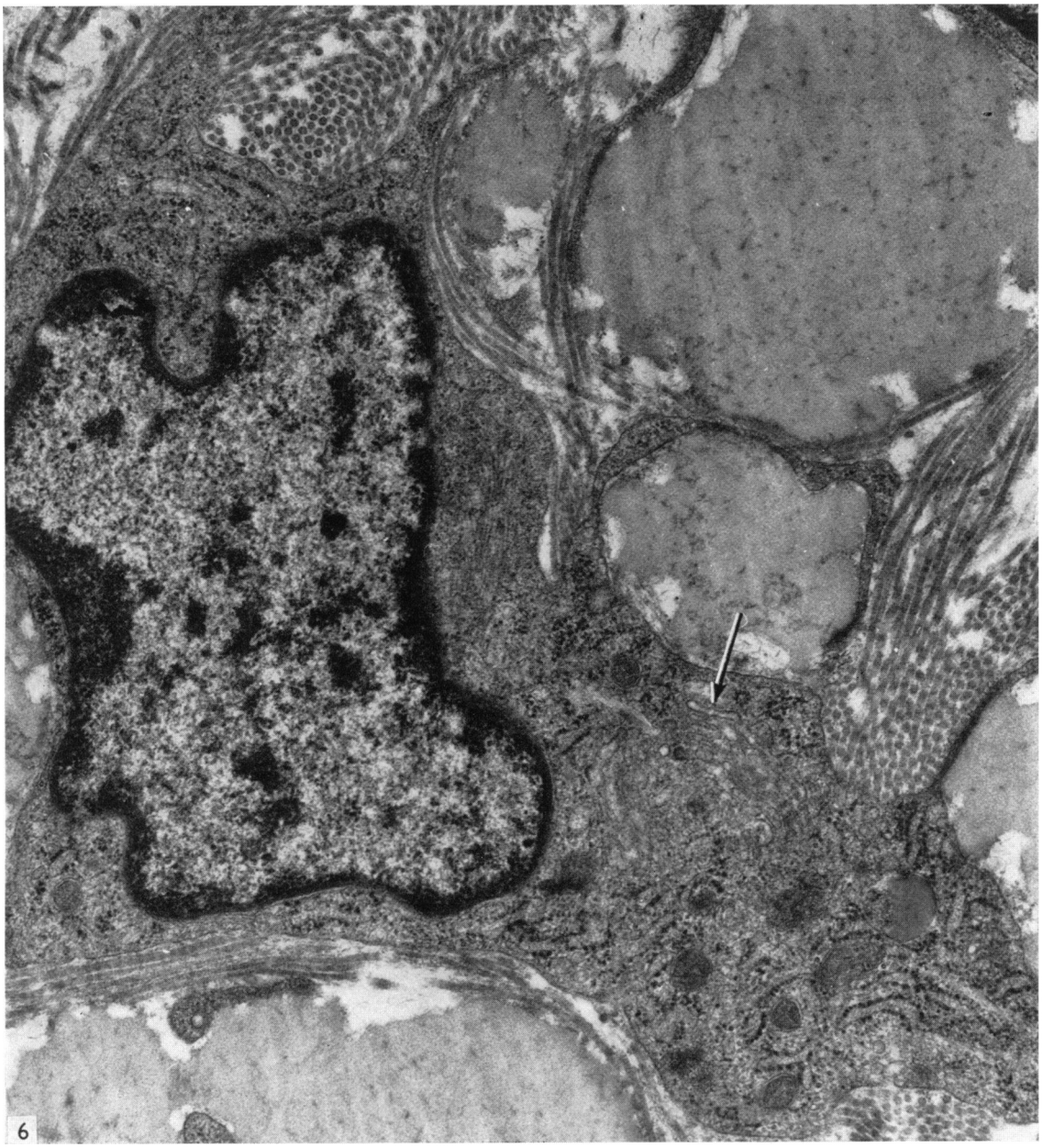

Fig. 6. Relaxed. The Golgi complex of this cell is well developed and this is typical of most of the cells (arrow). × 21 600.

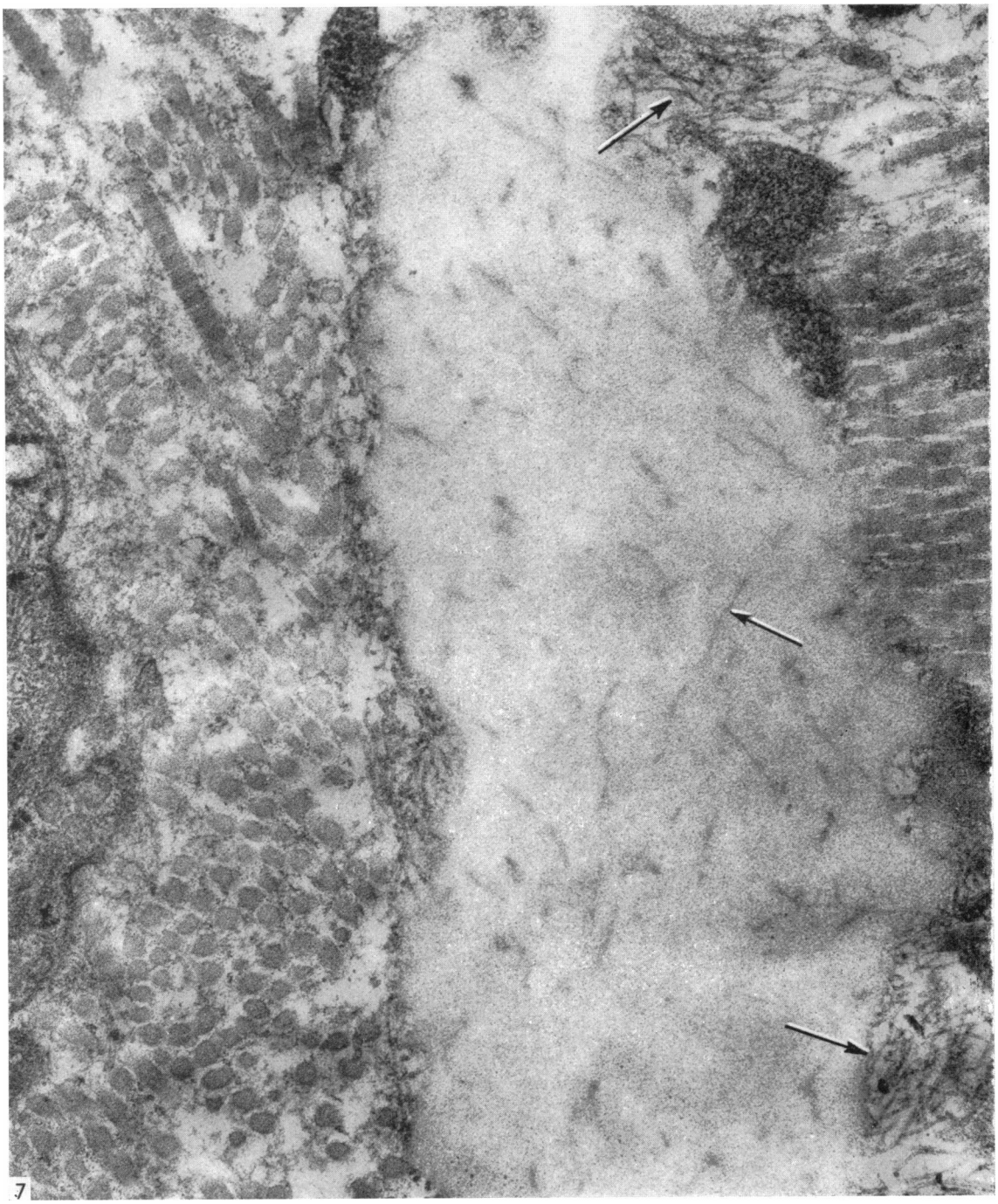

Fig. 7. Relaxed. Microfibrils (arrows) are seen on the periphery of the elastic fibres as well as within the fibre. × 35 500.

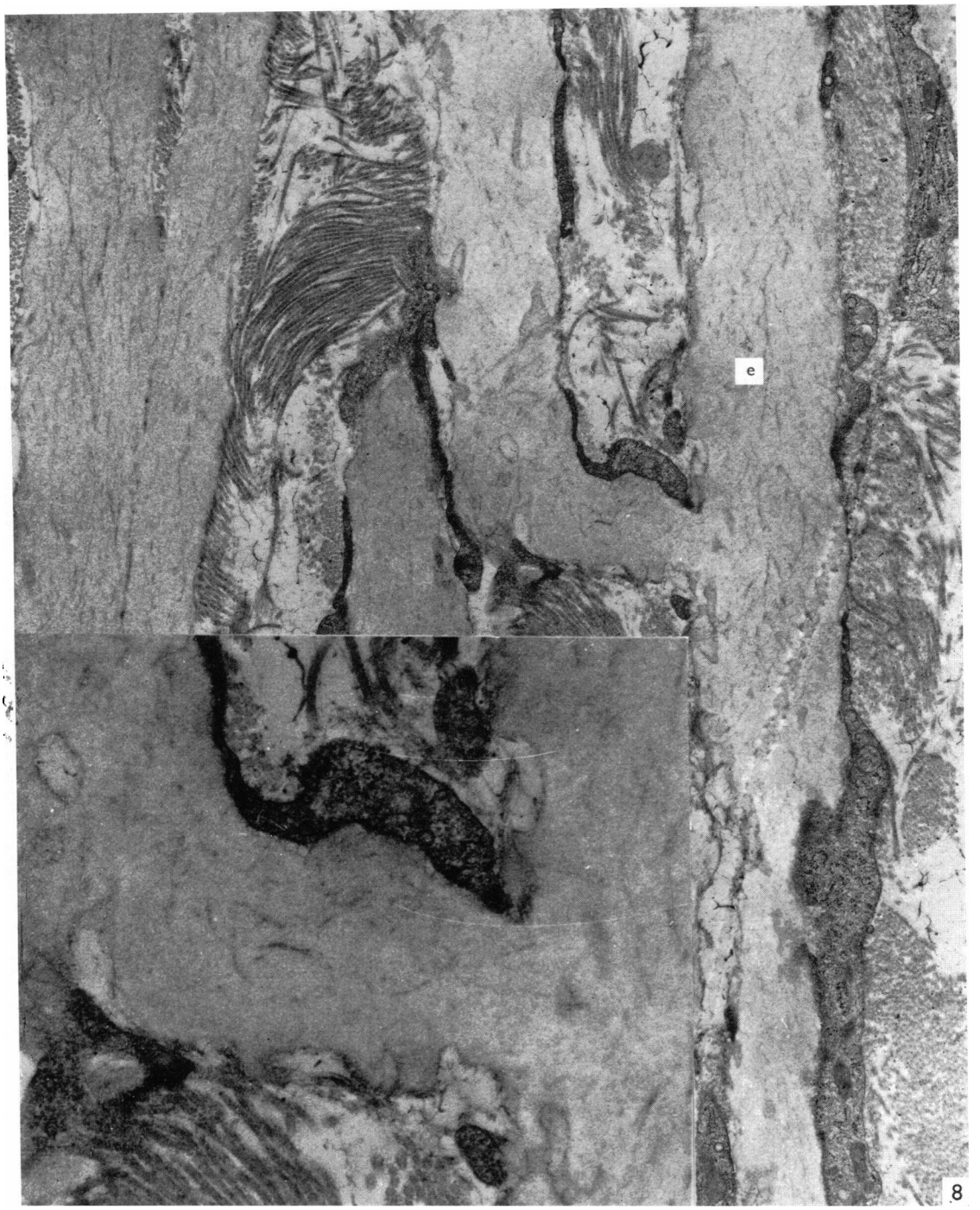

Fig. 8. Stretched. One elastic fibre (e) has split off at a right angle. ×11100. Insert. Stretched. Higher magnification to show that the appearances are not a result of tangential sectioning. ×27800.

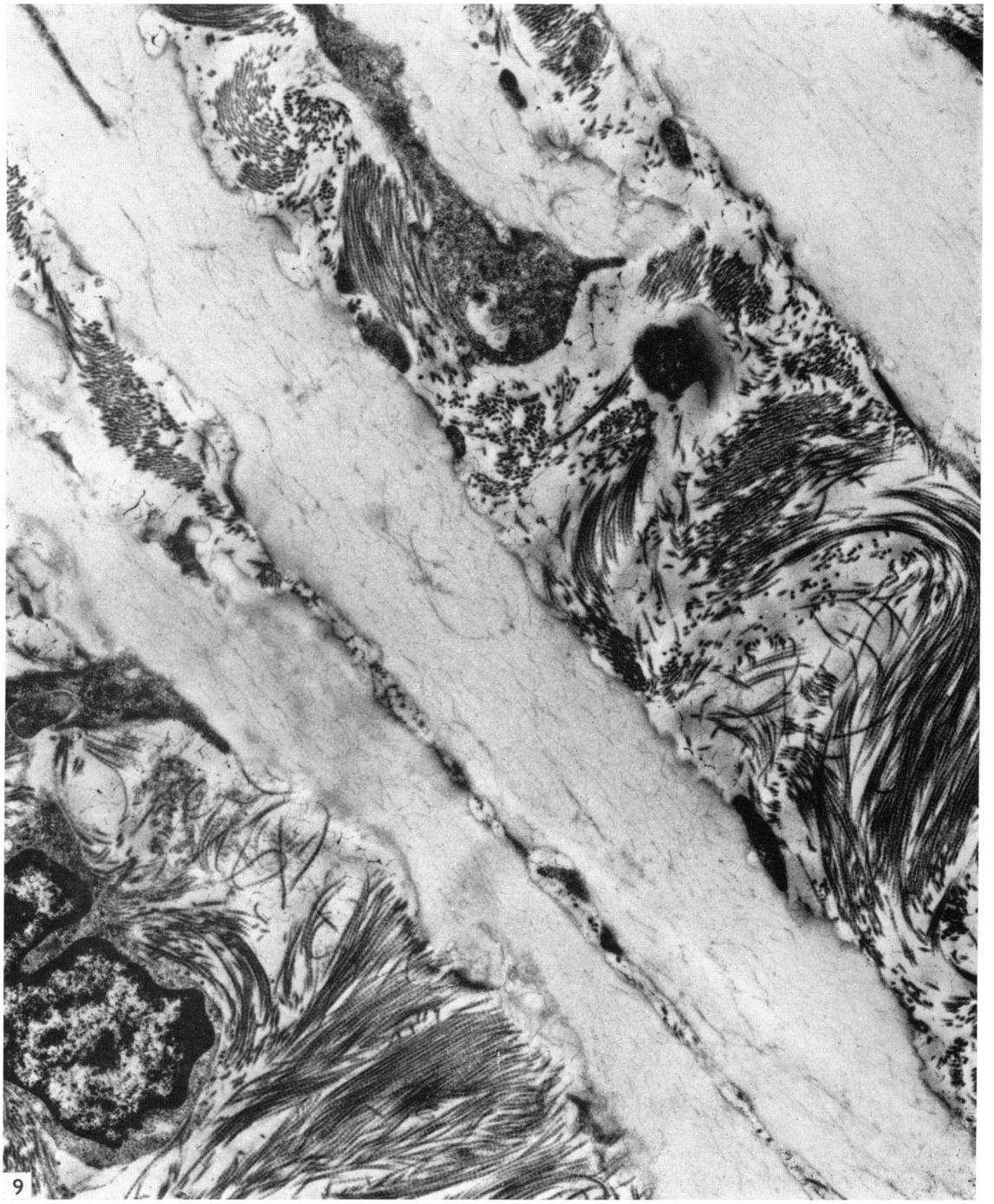

Fig. 9. Relaxed. Looping bundle of collagen fibrils, along with other randomly oriented collagen fibrils, typical of a relaxed vinculum. ×11000.

the typical collagen pattern, in which some of the fibres are seen looping between the elastic fibres and some are cut in various planes.

Stretched. Cross sections of the stretched vincula resembled those of the relaxed vincula in that some collagen still appeared to loop around some of the elastic fibres. However, most of the collagen fibrils now seemed to be cut transversely.

In longitudinal sections the long axes of the stretched elastic fibres remained parallel over longer distances than did the relaxed ones (Fig. 10). The collagen fibres were not as well oriented as the elastic fibres, though in the vinculum illustrated the collagen fibres mostly ran in much the same direction as the elastic fibres and the looping appearance had almost disappeared. Nevertheless there were many collagen fibrils that did not run parallel to the long axis of the vinculum, but even they seemed to be straighter and more orderly than those in the relaxed vincula. Some of the collagen fibrils were interwoven and were not pulled out into perfect alignment with the elastic fibres even when the vinculum was stretched to breaking point.

As a vinculum was stretched the elastic fibres changed in diameter as well as length. Thus in a relaxed vinculum, fibre diameters ranged between 0·7 and 4·8 μm, while in a fully stretched vinculum, the fibre diameters ranged between 0·1 and 2·7 μm.

The differences in the alignment of collagen fibres in relaxed and stretched vincula did not appear to be very great when their cross sections were compared. However in longitudinal sections there were distinct differences in the collagen of relaxed and stretched vincula. The cross sections showed that, even when the vinculum was fully stretched, there was still some interweaving of collagen fibrils around the elastic fibres. The longitudinal sections, however, showed that in fully stretched vincula most of the collagen fibrils were taut and were aligned with the elastic fibres in the long axis of the vinculum.

DISCUSSION

No doubt a most important function of the vincula is the delivery of a blood supply to the tendons, and this is in accord with the fact that many small vessels course through its superficial cellular layer. However, the size and structure of chick vincula indicate that they must have other functions.

When a vinculum is pulled out and allowed to relax it behaves like an elastic rubber band, yet its fibres are not simply elastic ones, but an interlacing network of collagen and elastic fibres. It is our hypothesis that the interweaving pattern of collagen around the elastic fibres acts as a 'check-rein' mechanism, the elastic fibres acting to absorb a rapidly applied force until the collagen becomes taut, and then the force is either transmitted to the bone or tendon or is simply resisted by the collagen.

Our hypothesis that collagen acts as a check-rein mechanism is supported by the work of Hoeve & Flory (1958) who performed force-temperature measurements on *ligamentum nuchae* before and after the removal of elastin with elastase. They compared the elastic properties of elastin to rubber and concluded that 'in contrast to rubber, the steep rise in stress at elongations near the maximum attainable

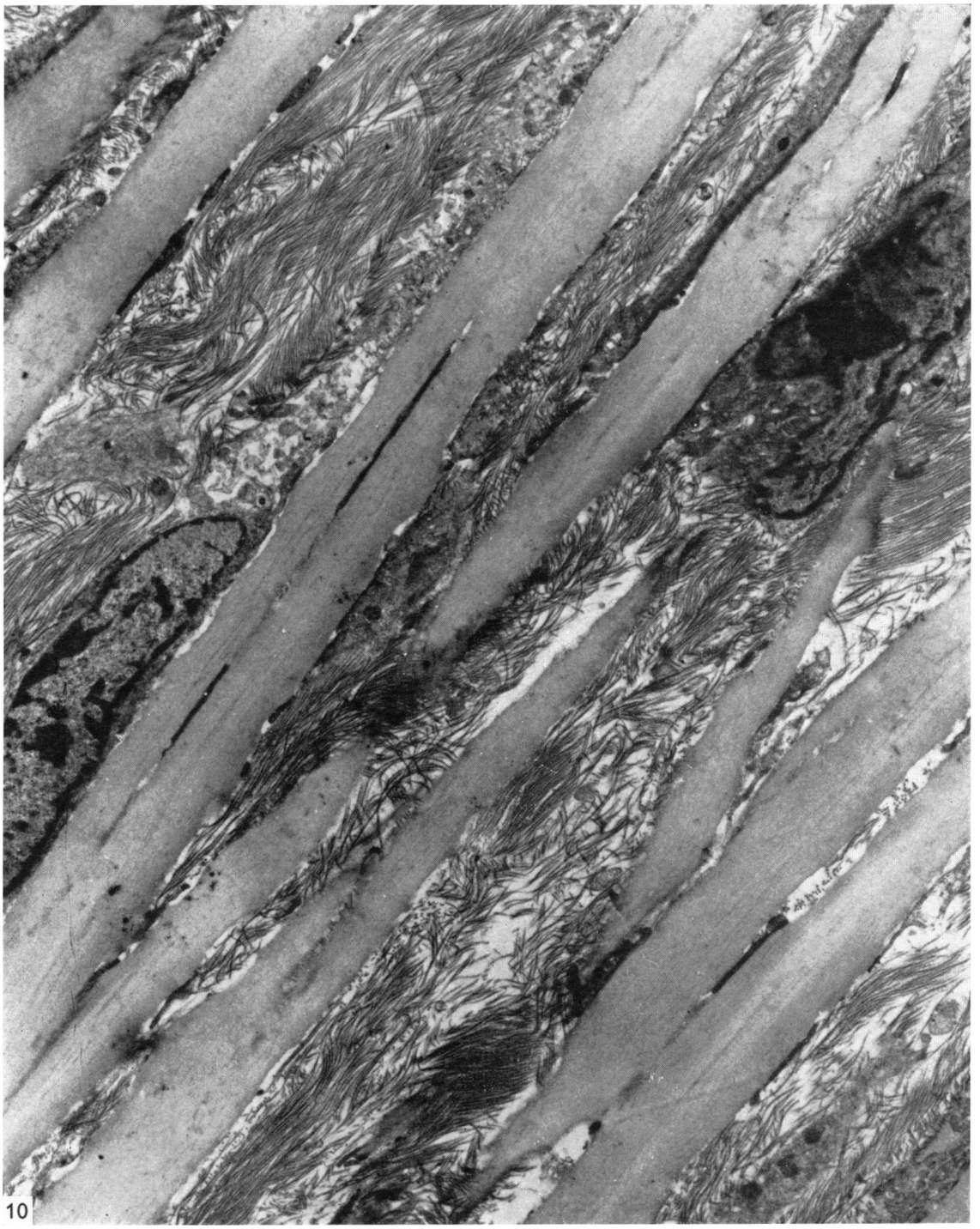

Fig. 10. Stretched. In longitudinal section the long elastic fibres are almost parallel. The collagen fibrils are largely oriented along the lines of tensile stress. × 10000.

length is not precipitated by crystallization, but by a permanently crystalline component (collagen) interwoven with the deformable (elastin) component'.

A recent report by Hoffman, Park & Abrahamson (1973) further supports our conclusions. They subjected *ligamentum nuchae, in vitro*, to varying sequences of enzyme and/or buffer treatments. They used collagenase, hyaluronidase+β-glucuronidase, trypsin and elastase. Then the specimens were stressed to failure. They concluded that the elastic fibre network prevented the 'alignment of the collagen fibre network which could result in irreversible changes in the dimensions and microstructure of the tissue'. Indeed, our morphologic study of an overstretched vinculum showed that alignment of the collagen fibrils did not really begin until the vinculum was about to rupture.

SUMMARY

From their mode of attachment and their elastic composition, it is clear that the vincula of the chick serve other functions besides that of carrying blood vessels to the digital flexor tendons within their synovial sheaths. Evidence is presented in support of the argument that elastic fibres bear the brunt of rapidly applied tensile forces and that the interweaving collagen fibres only become taut when the vincula are stretched to the limit and about to tear. Our hypothesis is that the collagen serves as a check-rein mechanism in an otherwise elastic structure.

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