FINE STRUCTURE OF PACINIAN CORPUSCLES
IN THE MESENTERY OF THE CAT

KATSUHIDE NISHI, CHIKAYOSHI OURA, and WAZIR PALLIE

From the Departments of Physiology, and Anatomy, Kumamoto University Medical School, Kumamoto, Japan, and the Department of Anatomy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. Dr. Pallie's present address is McMaster University, Hamilton, Ontario, Canada

ABSTRACT

Pacinian corpuscles in the mesentery of adult cats were fixed with either glutaraldehyde, osmium tetroxide or permanganate solutions by close intra-arterial injection through the mesenteric artery, and were processed, after electron staining and Epon embedding, for electron microscopy. Better resolution of the corpuscle's ultrastructure was obtained than available heretofore. The myelinated segment of the corpuscle contains blood vessels separated from the axon by collagen fibers and 3 to 4 layers of lamellae. No blood vessels are found in the central core, though access from the vessels is afforded by diffusion through the "cleft" of the inner core. Two cell types are discernible in the inner core hemilamellae; the "clear cells" in which pinocytotic vesicles and organelles abound and reflect the greater metabolic activity of these cells, in contrast to the "dark cells." The ultraterminal is ellipsoidal in form with projections into the "cleft" which give this portion an irregular appearance in section. The terminal and ultraterminal are packed with mitochondria, and "synaptic" vesicles are seen in the ultraterminal. The innermost laminae of the inner core cells are in close apposition to the terminal and break their regular pattern of hemilamellation to surround the small ultraterminal projections at the apical part of the corpuscle.

INTRODUCTION

In spite of the great interest which has been shown by light microscopists of Pacinian corpuscle morphology in the past and in recent years (2, 6, 13, 14, 15), only a few studies have appeared on the electron microscopic appearance of this structure. Pease and Quilliam (11) have shown that the corpuscle has an outer zone, in which there is a framework of lamellae arranged concentrically, and a core, in which lamellae surround the nonmyelinated nerve terminal bilaterally and show characteristic specializations unrecognisable by light microscopy. However, their studies as well as those of Polacek and Mazanee (12) suffer from the use of methacrylate embedding which does not permit clear resolution of fine structures such as is afforded by the employment of epoxy resin embedding in conjunction with heavy metal electron stains. The fine structure of the nerve terminal and of the lamellar cells that enfold it in mature corpuscles has significance in physiological considerations. Refinements of preparation are necessary if this fine structure is to be studied in detail satisfactorily. The present study was carried out to reexamine the fine structure of Pacinian corpuscles in the mesentery of adult cats, and to attempt to elucidate functional relationships such as the difference in directional sensitivity to mechanical stimuli in the nonmyelinated nerve terminal of the corpuscle demonstrated recently (3, 4, 9).

MATERIALS AND METHODS

In order to overcome difficulties in obtaining satisfactory fixation of the core region of corpuscles from adult cats, close intra-arterial perfusion methods with solutions containing fixative were employed. Adult cats were anaesthetised by intraperitoneal injection of 5-ethyl-5-*iso*amyl barbiturate (50 mg/kg body wt), the abdominal cavity was opened widely by a midline incision, and the small intestine and its mesentery were exteriorised and exposed. At the root of the mesentery, the superior mesenteric artery was carefully isolated from the vein and accompanying nerve fibres and was cannulated with a polythene tube, to the free end of which an injection syringe filled with fixative solution was connected. Perfusion was achieved through the syringe by manual pressure, and the procedure was completed within a 2 min period. The fixative used was either 1% glutaraldehyde buffered with Veronal-acetate at pH 7.2, 1% osmium tetroxide buffered with S-collidine at pH 7.2, or 3% potassium permanganate buffered with Veronal-acetate.

After preliminary fixation, a piece of the mesentery, approximately 3 cm² and containing several corpuscles, was excised and attached on a lucite plate which was then immersed in fresh 1% glutaraldehyde solution. Corpuscles were dissected from the mesentery with fine forceps and needles under a binocular microscope. Subsequent postfixation was carried out by one of two alternative methods: in the one, the corpuscles were fixed in cold 1% or 2% osmium tetroxide buffered in either Veronal-acetate or S-collidine for approximately 4 hr; in the other, the specimen was fixed for approximately 1 hr in cold 3% potassium permanganate in Veronal buffer. After postfixation, the specimens were washed briefly in their respective buffer solutions and dehydrated within a 12 hr period in a series of increasing concentrations of ethanol. In most instances, block staining with a saturated solution of uranyl acetate dissolved in 80% ethanol was incorporated into the dehydration process. After embedding in Epon 812, ultra-thin sections were obtained on a Porter-Blum microtome with glass knives. The sections were then stained in basic lead citrate and carried through for examination in the Hitachi model HU-11A electron microscope. Electron micrographs were taken at calibrated magnifications ranging from 2,000 to 20,000 times and photographically enlarged as desired.

OBSERVATIONS

Proximal Region of the Corpuscle (the Preterminal Myelinated Segment)

This portion lies external to the inner core of the corpuscle. The Pacinian corpuscle is usually ellipsoidal in shape, and each corpuscle is supplied by a single myelinated nerve fiber (14). In typical corpuscles examined, a single myelinated nerve fiber, 5 to 8 μ in diameter, enters the proximal pole of the corpuscle and takes a sinuous course in the first quarter or first third of its intracorpuscular length. In the vicinity are several blood vessels, some of them having smooth muscle fibers, surrounded by several layers of lamellar cells (Fig. 1). In cross-section, in the region distal to the intracorpuscular node of Ranvier, the axon is surrounded by thick bundles of collagen fibers, most of which are orientated parallel to the longitudinal axis of the axon, and by interspaced concentric layers of lamellar cells which bridge the 3 to 10 μ interval separating the capillary from the nerve fiber. The periaxonal spaces are wider than the pericapillary ones, the lamellae being knit more tightly in the vicinity of the latter (Fig. 1). Cross-sections of the corpuscles in this region bear a resemblance to the cross-section of the capsule of the muscle spindle (5, 7)·

In both longitudinal and transverse sections at high magnifications, the lamellar cells appear as thin sheets of cytoplasm with cell membranes, on either or both sufaces of which basement membranes are demonstrable (Fig. 2 *a*). These cells contain small mitochondria, ribosomes, and occasionally a few pinocytotic vesicles. The interlamellar spaces are occupied by irregularly orientated collagen fibrils and by amorphous substance. Some adjacent margins of neighbouring cells show "junctional complexes" between cell membranes as illustrated clearly in the multiple junction of cells in Fig. 2. Cross-sections of the axon with a small number of mitochondria and well preserved neurofilaments do not differ in appearance from a cross-section of ordinary myelinated nerve fibers. The fiber itself, having lost its surrounding myelin sheath, thence enters the central core region.

Central Region of the Corpuscle (the Terminal Nonmyelinated Segment)

In transverse sections through the central region of the corpuscle the nonmyelinated nerve terminal is seen surrounded by inner core cells arranged symmetrically to form hemilamellae arranged on each side of a longitudinal cleft, while more externally the outer lamellar cells of relatively high electron density are arranged concentrically in more widely spaced lamellation.

FIGURE 1 Transverse section through the pre-terminal myelinated region of a Pacinian corpuscle from adult cat. The myelinated nerve fiber (*NF*) contains scattered mitochondria and is ensheathed by Schwann cytoplasm (*S*). Lamellar cells (*LC*) and nuclei (*N*) lie concentrically arranged, and longitudinal collagen fibrils (*CF*, arrow) are present in the interlamellar spaces. The capillary (*C*) is separated by a 4.5 μ space from the axon, and the interval is occupied by lamellar cells and interlamellar contents. Fixation: 1% OsO_4 buffered with S-collidine. $\times$ 8000.
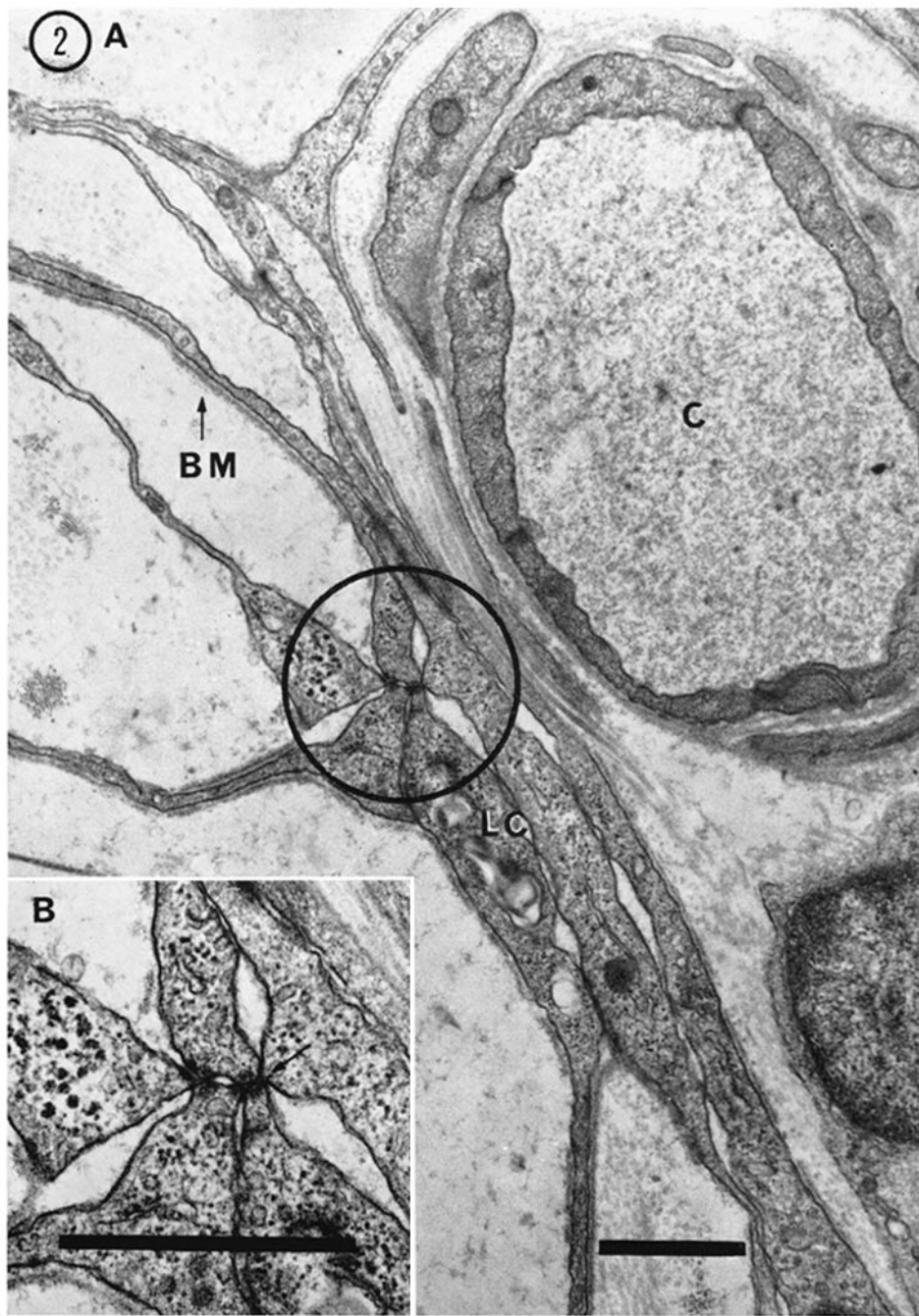
Figure 2 *a* Portion of previous figure at higher magnification showing lamellar cells (*LC*) with basement membranes (*BM*) which are visible on both surfaces of these cells, and collagen fibers arranged irregularly in the interspaces. × 20,000. Figure 2 *b* Shows further magnification of area encircled in Fig. 2 *a*, to demonstrate "junctional complexes" between a few lamellar cells (arrow). × 42,000.

The nonmyelinated nerve terminal is not circular but elliptical in cross-section, with its long axis placed in the plane of the longitudinal cleft of the inner core. A striking feature of the terminal is the accumulation of mitochondria layered below the surface membrane, and an amorphous or finely fluffy central zone in which several fine neurofilaments are demonstrable. Short, tapered or club-like projections of the terminal, of a higher electron density than the axoplasm, protrude on one or both sides into the "cleft (Figs. 3 and 4)." Though these projections have been considered artefacts by others (11), they were present consistently in our well preserved specimens after different fixation and preparation techniques. Though these processes of the nerve terminal do vary somewhat in shape and length, they are a normal feature of the terminal at least in mature corpuscles. Also seen within the axoplasm are vesicles, 300 to 500 mμ, lying near the axolemmal membrane and in the vicinity of short projections into the "cleft (Fig. 4)." These vesicles are indistinguishable from the synaptic vesicles of mammalian ganglia (11, 14) and of muscle spindle terminals (5, 7).

The inner core of the Pacinian corpuscle consists of closely packed lamellar cells in hemilamellar arrangement, and the outermost regions have thicker lamellae with more cytoplasm and contained nuclei. Subject to the reservation that perfusion fixation may vicariously produce fluid dislocation in adjacent cells, "clear" and "dark" cells are distinguishable with corresponding lamellar processes unevenly distributed throughout the "core" region; these cells are layered in a complex manner with their cytoplasmic arms forming the flattened hemilamellar sheets of the inner core. In addition, the clear and dark cells extend their cytoplasmic arms along the "cleft" (Figs. 5 a and b). Cell organelles such as mitochondria, Golgi apparatus, pinocytotic vesicles, and endoplasmic reticulum are more abundant in the clear cell and are best seen in the vicinity of the flattened nucleus and the larger cytoplasmic arms of the lamellar cell (Fig. 5). High magnifications reveal that the core lamellar cell possesses a basement membrane on both surfaces and several cell organelles, the pinocytotic vesicles being a dominant feature of the clear cells (Fig. 6) and suggesting a nutritive role besides a supportive role for these cells.

The interlamellar spaces of the central core contain amorphous substance and, contrary to previous observations (14), do contain collagen fibrils chiefly orientated in the long axis of the corpuscle. Nearer the ultraterminal end, the spaces are reduced greatly and their contents scanty.

The intermediate zone or "space" between the inner core and the outer core is sharply defined in mature corpuscles and abutting on it are several nuclei; the nuclei on the exterior of it belong to the outer core cells, and those on the interior of it belong to the inner core cells; the "space" itself contains amorphous material and collagen fibrils.

Ultraterminal Region of the Corpuscle (the Apical Segment)

The distal end of the terminal nerve fiber is expanded irregularly and angular and continues on to bi- or trifurcation (Fig. 7). The fine projections of the terminal, packed with several mitochondria and a few vacuoles, are intimately enfolded by the innermost core cell processes.

At this level, core lamellar cells are not arranged bilaterally and symmetrically, but surround the nerve terminal irregularly. The clear cleft seen in the central part of the corpuscle is not observed in the ultraterminal region. Instead, narrow extralamellar spaces extend toward each ultraterminal process and cause the core lamellar cells to envelop these processes in separate concentric groups, usually 3 to 4. When the ultraterminal is branched, each branch is less than 1 μ in diameter and about 10 to 20 μ long. The tips of these terminals show a circular outline and contain several mitochondria, but synaptic vesicles were not found here, in contrast to the more proximal part of the nonmyelinated segment (Fig. 8).

The core lamellar cells abut on the ultraterminal processes and contain pinocytotic vesicles about 100 μ in diameter (Fig. 9).

In cross-sections of the corpuscle at this level, the inner core still shows the two cell types, dark and clear, the latter being packed abundantly with organelles. The outer core cells are demarcated by the "space" from the inner core, and this delineation is emphasised by bundles of collagen fibrils in concentric disposition.

From light microscope studies it has been

FIGURE 3 Cross-sectional view of central region of Pacinian corpuscle through the nonmyelinated nerve fiber (*NF*), seen as an elliptical outline, containing several mitochondria and a short projecting process (*P*). The latter extends into the *cleft* between the hemilamellae of the inner core. The clear cells (*CC*) and the dark cells (*DC*) are seen in the inner core which is demarcated clearly from the outer core at the top right hand corner. Fixation: KMnO₄ buffered with veronal-acetate. × 6600.
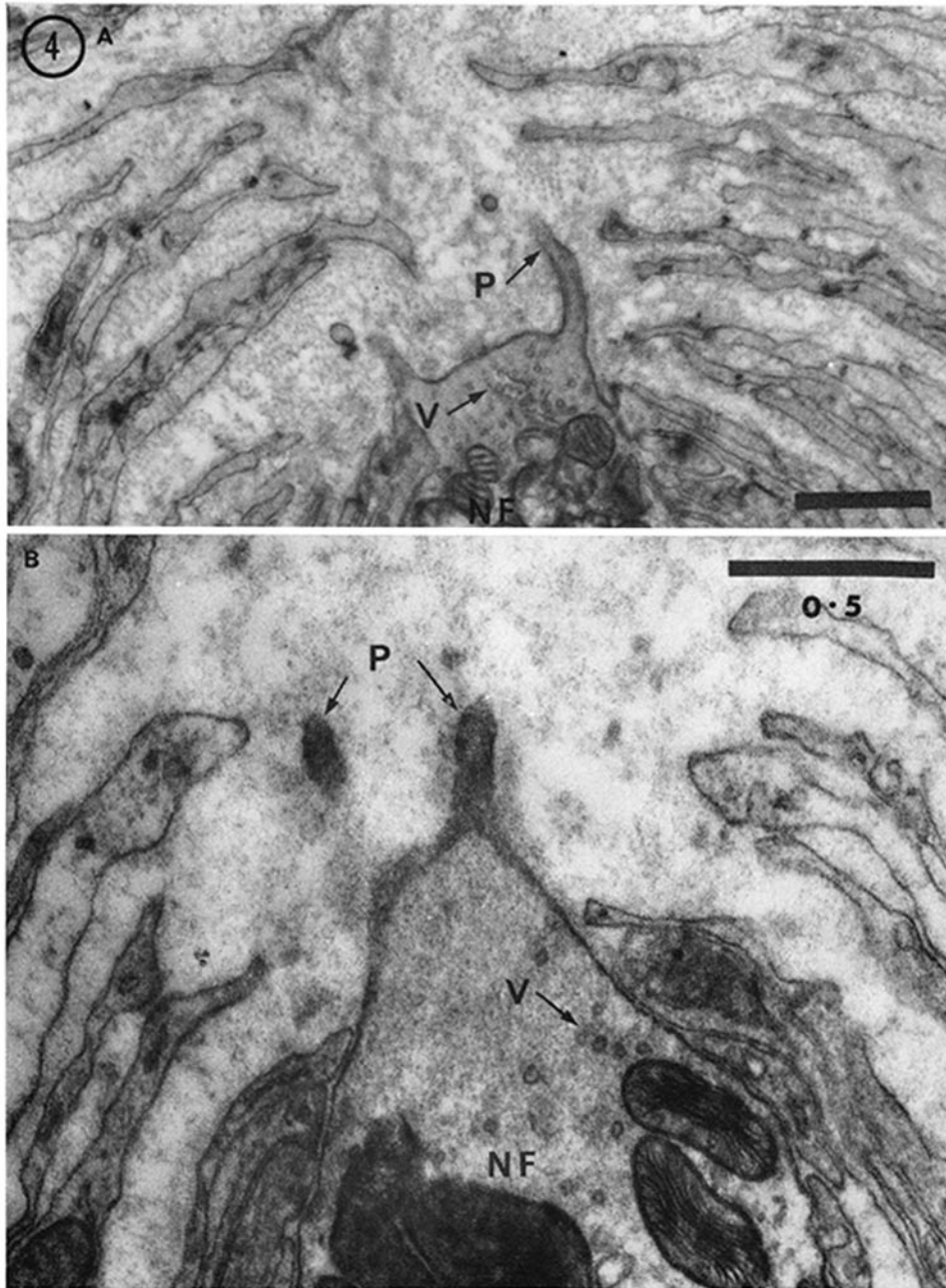
FIGURE 4 *a* and *b*   Nerve terminal (*NF*) and "cleft" of Pacinian corpuscle. (*a* and *b* were obtained from different animals). Short projections of axoplasm (*P*) project into the "cleft." The axoplasm in the terminal contains many small vesicles (*V*) and is packed with mitochondria. *a*, × 18,000; *b*, × 52,000.
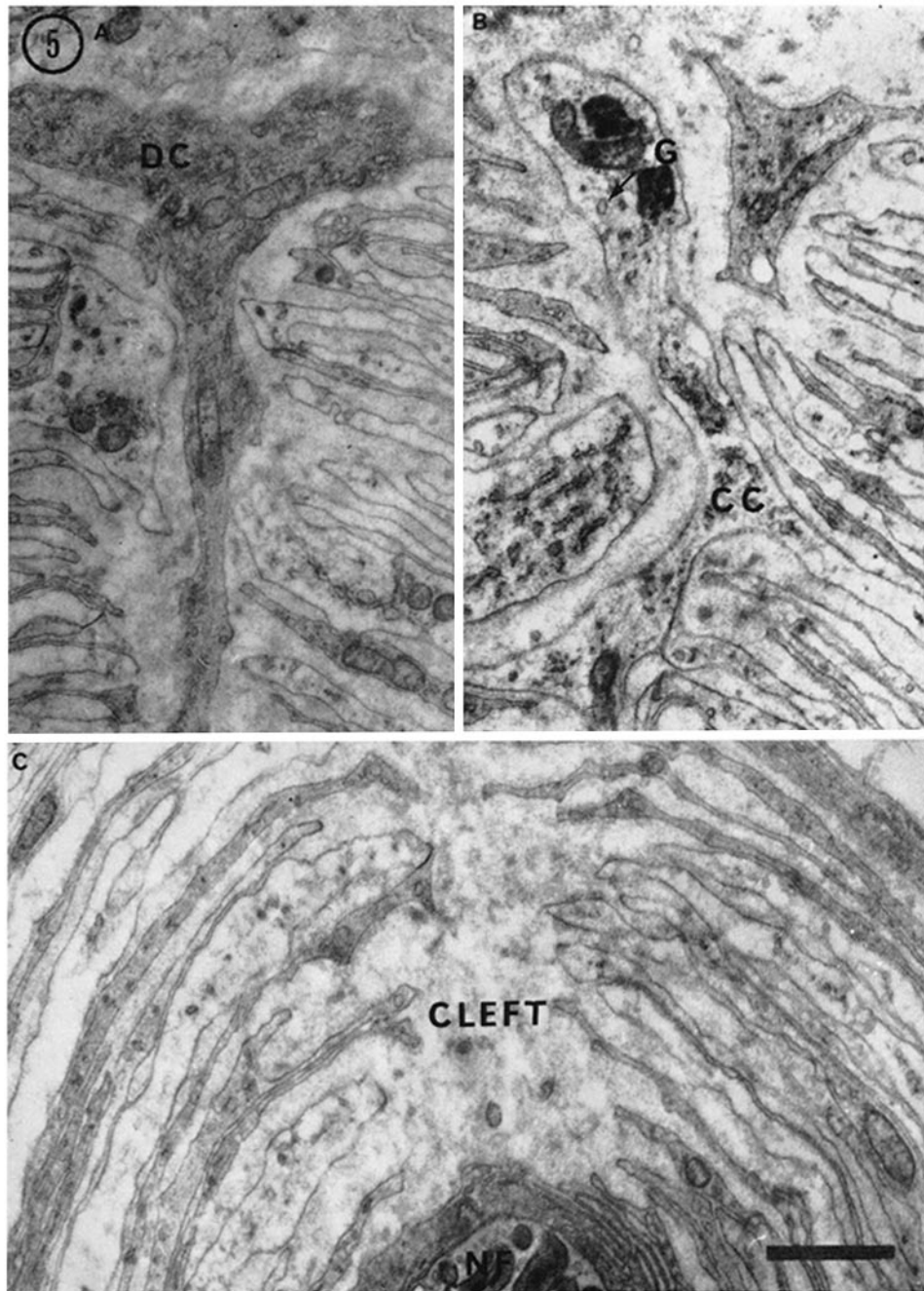
FIGURE 5 a, b, and c   Transverse sections of central core and "cleft." In a and b, massive cytoplasmic arms are seen extending into the cleft; in a, the process extends from a dark cell (DC), and in b it extends from a clear cell (CC). These arms are extensions of cells in the periphery of the inner core. In contrast, in c a wide unoccupied cleft is seen leading to the nerve terminal (NF). Fixation: 3% KMnO₄ in veronal buffer. × 17,000.
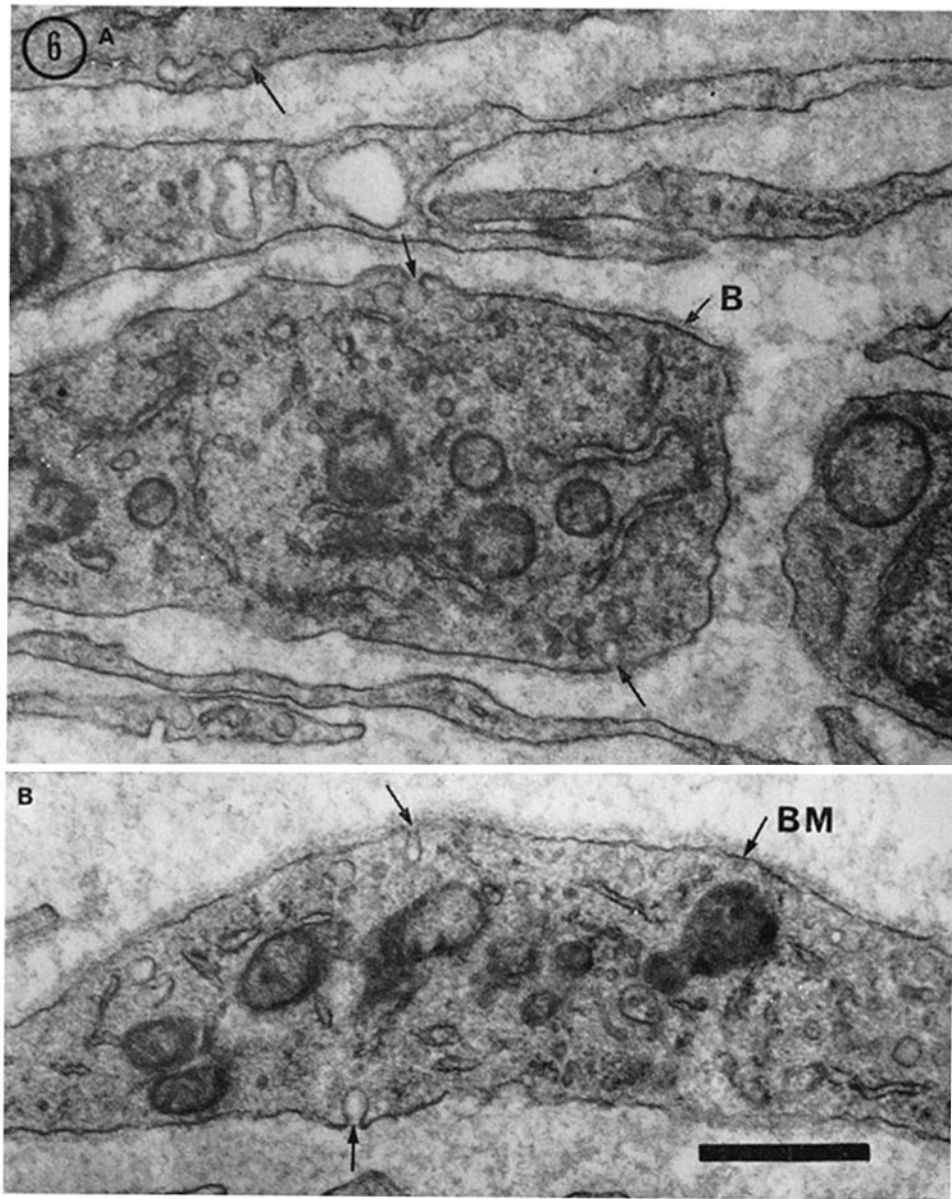
FIGURE 6 *a*, *b* and *c* High power views of central core lamellar cells. The cells are lined by basement membranes (*BM*), and a number of pinocytotic vesicles (arrows) are present on both surfaces of the cell. Within these cells the mitochondria, endoplasmic reticulum, and small vesicles are well preserved. × 45,000.

claimed that branches of the nerve terminal end in round osmophilic swellings 8 to 12 μ in diameter (13). Such large terminals have not been observed in this study; instead, from a study of successive ultra-thin sections, it is more apt to describe the branched nerve fibre terminal as

gradually decreasing its diameter into an attenuated termination.

DISCUSSION

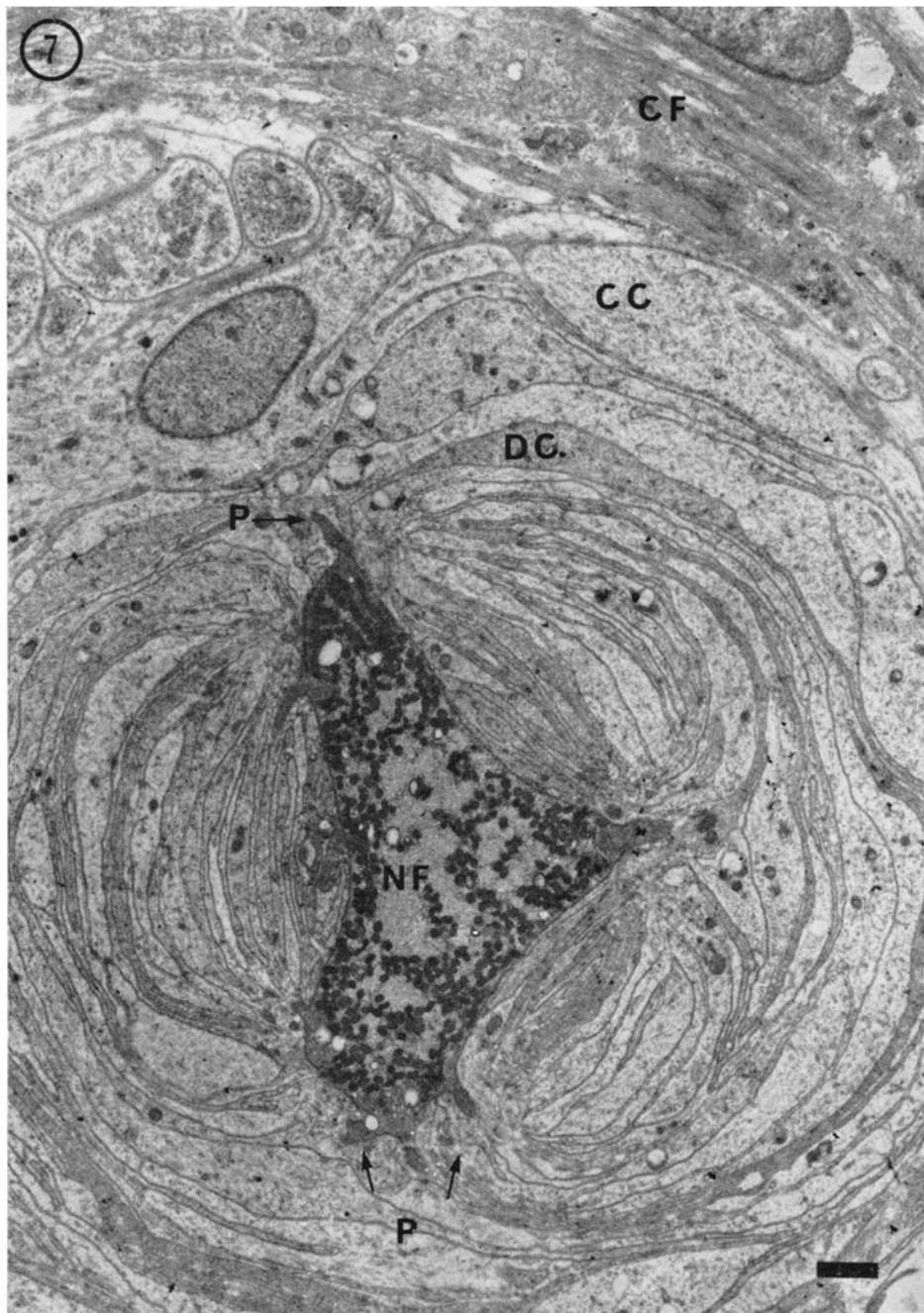Physiological observations made by Diamond et al. (1) and Nishi (8) that impulses elicited from

FIGURE 7 Low power view of the ultraterminal region of a Pacinian corpuscle. Note the trifurcated form of the termination with short projections (*P*) at each corner and the subdivision of the hemilamellae with no evidence of the "cleft" as seen in sections at the lower levels. Clear cells (*CC*) are distinguishable from dark cells (*DC*). The periphery of the core is ringed by thick collagen fibers (*CF*). *NF*, nerve terminal. Fixation-1% OsO$_4$ in veronal buffer. × 6000.
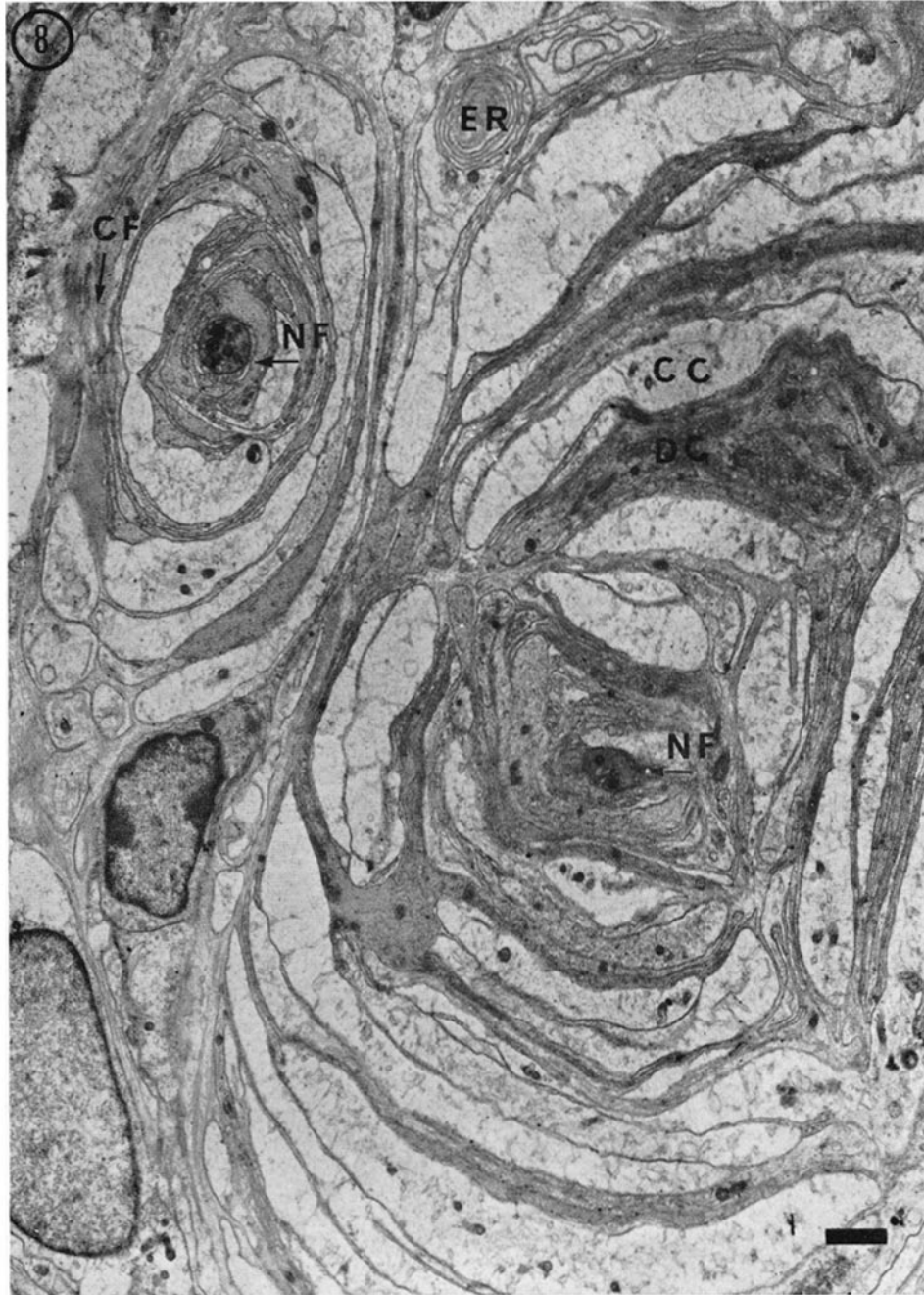
FIGURE 8   The apical portion of a Pacinian corpuscle showing the extreme tips of the branched ultra-
terminal. Two of these are seen (*NF*) surrounded concentrically by core lamellar cells. Collagen fibers
(*CF*) are seen in the vicinity where extralamellar spaces occur. Clear cells (*CC*) with endoplasmic retic-
ulum (*ER*) and dark cells (*DC*) are shown and reflect fluid dislocations which make unequivocal inter-
pretations of these cells difficult. Fixation: 1% OsO₄ in veronal buffer. × 5250.
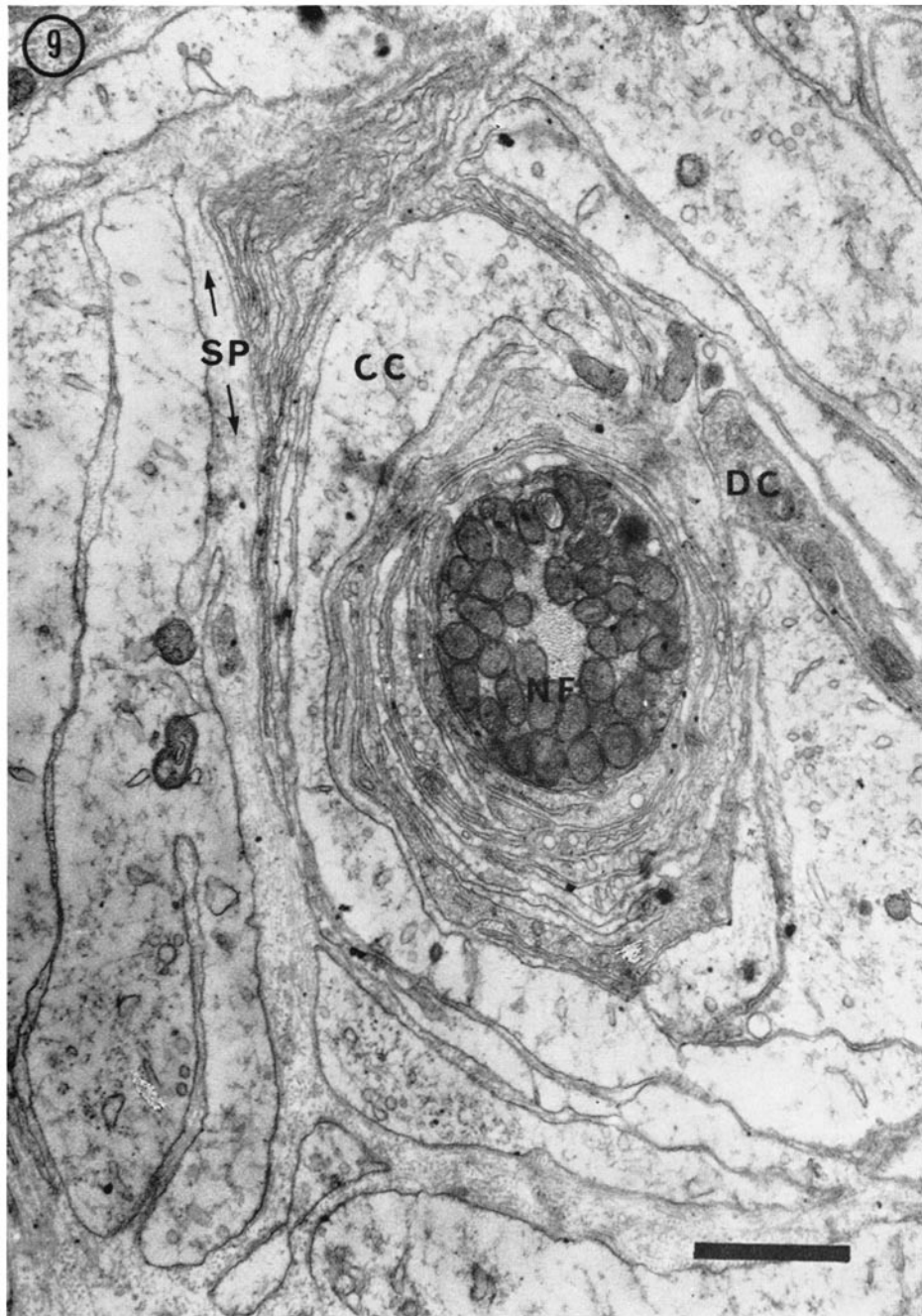
FIGURE 9   A higher magnification of a single filament forming the tip of the ultraterminal seen in cross-section. The tapering nerve fiber (*NF*) is filled with mitochondria and centrally located neurofilaments, but vesicles are absent. Many pinocytotic vesicles are observed in the core lamellar cells which here intimately surround the ultraterminal. The more peripherally located interlamellar spaces (*SP*) are occupied by collagen fibers and dark cells (*DC*). Clear cells (*CC*) are again discernible though the possibility of fluid dislocations by fixation cannot be entirely discounted. Fixation: OsO$_4$ in veronal buffer. Scale line = 1 μ. $\times$ 20,000.

the Pacinian corpuscle were abolished by perfusing it with Na-free solution, support the postulation of Pease and Quilliam (11) that the cleft in the central core region is the metabolic exchange route for the nerve terminal. In the present experiments, the very satisfactory fixation of the inner core achieved by close intra-arterial perfusion of the cat mesentery gives further confirmatory evidence.

Cross-sections of the Pacinian corpuscle reveal the difference in the arrangement of the concentric outer core lamellar cells and the hemilamellar configurations of the inner core. Though the former have a generally higher electron density, they otherwise have an appearance similar to that of the latter in regard to contained organelles and lining basement membranes; furthermore, collagen fibrils are present between the lamellae of both regions, contrary to previous observations (11). Whether or not the outer and inner cores have a common embryological origin is thus not determinable on such morphological grounds alone. We have described the occurrence of two cell types within the inner core, the clear and the dark cell. The former, because of its greater content of organelles and pinocytotic vesicles, is likely to have an active metabolic role as opposed to a purely supporting role, though the difference between the two cell types may be purely functional and not developmental. The close relationship of such cells to the distalmost part of the nonmyelinated terminal and its ultraterminations, membrane to membrane, provides for ready exchange either for purely metabolic functions or transduction of action potentials. Continuity of the Schwann cell lining of the nonmyelinated terminal into its more distal investing inner core cells was not established in this study, which inclines one to believe that the two are probably distinct cell types. This is in contrast to the descriptions of genital end-bulbs (10) where the "laminar" cells have been considered to be modified Schwann elements. It is tempting to suggest that the several forms of cellular encapsulation of end-organs is determined around a "naked" axon reaching its termination, by the epithelial or connective tissue environment in which it finds itself, and that the axon is modified for functional and/or metabolic purpose as we find it in the formative end-organs. The axoplasm of the nerve terminal in both Pacinian corpuscles and muscle spindles (5, 7) contains a large number of mitochondria, neurofilaments, and "synaptic" type vesicles; these are characteristic of sensory nerve endings in general. The nerve terminal within the central core of the Pacinian corpuscle is not circular but slightly flattened or ellipse-shaped, with the long axis corresponding to the plane of the cleft between the inner core lamellae. Directional sensitivity to mechanical stimulation has been demonstrated recently (3, 9), in that a receptor (depolarizing) potential on compression stimulation was converted to hyperpolarization to gradually increasing compression, after rotation of the corpuscle on its long axis, and is explained on the basis of the shape of the terminal and the hemilamellar arrangement. In the present study, by marking the point of compression with a fine needle in such electrophysiological experiments, we have confirmed by subsequent electron microscopy that compression directed along the short axis of the ellipsoid terminal produced depolarization, and vice versa.

In mature Pacinian corpuscles, tapered or blunt-ended processes of the terminal of a relatively high electron opacity protruded into the "cleft" of the inner core. The length and shape of these protrusions varied from one specimen to another, but there is no doubt that these processes are not artefactual processes, which is an interpretation given to them by previous investigators (11). We have demonstrated morphologically that the ultraterminal region is irregular in outline in cross-section, being angulated or trifurcated, and contains several mitochondria. Different fixation methods and preparation techniques as well as different planes of section confirm that the ultraterminal is not roundly swollen into a bulbous extremity. The bi- or trifurcation or, indeed, multiple division of the ultraterminal with some disorganisation of the arrangement of the central core hemilamellation to form concentric lamellae around these ultraterminals, may confer a sensitivity of the apical portion of the corpuscle to stimulation from all directions, though it is difficult to envisage so small and selective a natural stimulus.

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