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Effect of Age on the Structure of Meissner Corpuscles in Forepad Digital Pads of Mice

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ABSTRACT

EFFECT OF AGE ON THE STRUCTURE OF MEISSNER CORPUSCLES IN FOREPAW DIGITAL PADS OF MICE

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Meissner corpuscles in forepaw digital pads of albino mice were examined by light and electron microscopy to determine structural age-changes. After 1.5 months of age, no intraepidermal nerve fibers were seen extending from corpuscles. From young (1.5-6 months) to middle age (9-15 months), corpuscles became larger and more complex, gaining more horizontally arranged terminals, associated lamellae and connective tissue. At old age (18-26 months), corpuscles became small and lobulated, appearing disorganized. There was no loosening of the corpuscle-epidermal interface. An increase in collagenous connective tissue and basal lamina duplication occurred with advancing age. Ultrastructural age-changes consisted of disorganization of the axonal organelles, rounding of the axon profiles with loss of axonal processes, regression of the perineural capsular epithelium, attenuation and loss of lamellae, loss of caveoli, mitochondrial degeneration and lipofuscin accumulation.

Intraepidermal terminals were thought to be present only during the period of corpuscle development which extends only to include age 1.5 months. Corpuscle growth and maturation until middle age possibly indicated sensitivity compensation for loss of corpuscles and innervating axons. At old age, the disruption of corpuscle

structure, especially with loss of axonal processes and horizontal arrangement of terminals, likely dampened the sensitivity of Meissner corpuscles, and raised their threshold to stimuli, perhaps making them nonfunctional. An increase in collagenous connective tissue was thought to have a significant effect on the physical transmission of pressure waves to the transducer. The significance of mitochondrial degeneration and lipofuscin accumulation was considered. Atrophic corpuscles seen in abundance by old age probably occurred as a consequence of age-changes in axonal transport promoting distal axonopathy.

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EFFECT OF AGE ON THE STRUCTURE OF MEISSNER CORPUSCLES IN FOREPAD DIGITAL PADS OF MICE

by

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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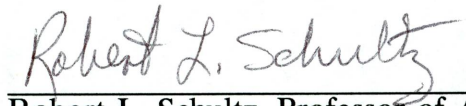

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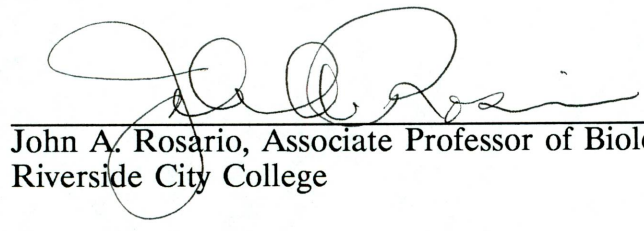

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All knowledge comes to us through our sense organs. Our simplest motor acts are initiated through sense organs; our most complex ones are controlled by means of them.

Theodore C. Ruch

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INTRODUCTION

Organisms with integument have a spectrum of cutaneous mechanoreceptors with variety and complexity depending on the higher order of species' development (Ruch, 1979; Somjen, 1983 Klauer, 1986). The vibrotactile sensations elicited by stimulation of rapidly adapting, lamellated mechanoreceptors, such as Meissner corpuscles, provide exteroceptive input for the body to interact with the external environment. As the organism ages, mechanoreceptors undergo changes which affect their response to stimuli (Spencer and Ochoa, 1981).

The Meissner corpuscle is the focus of this dissertation. Past research efforts on this corpuscle and closely related subjects are reviewed first. With pertinent background information, the present investigation of structural changes in aging murine corpuscles is then described, and the findings discussed relative to current concepts.

I. The Meissner Corpuscle

In the past century, many papers have been published that incorporate the Meissner corpuscle in their discussion, but no review has been written pertaining solely to this corpuscle. In the last two decades since a detailed summary of digital touch corpuscles written by Cauna (1956) which included Meissner corpuscles in the discussion, electron microscopy has greatly increased understanding about the structure of mechanoreceptors. Recent techniques of freeze-fracture have made it possible to begin analyzing ultrastructural aspects that are theorized to be an integral part of the mechanoelectric transducer apparatus. The recent investigative interest

in transduction mechanisms has made a complete understanding of the structure of mechanoreceptors extremely important.

It was initially believed that Meissner corpuscles were unique to man. Over the last two decades, other mammals have been found to have Meissner corpuscles. This has made research efforts more feasible by not having to rely only on human tissue, and, consequently, the volume of work has expanded tremendously. It is the purpose of this review to summarize work pertaining to Meissner corpuscles and put into perspective what is known of this mechanoreceptor. Areas of investigative focus that are emerging will be presented as well.

Meissner corpuscles were first described by Wagner and Meissner in 1852. The literature from the latter half of the nineteenth century and the first half of the twentieth century have already been reviewed by Cauna (1956). The present discussion will cover material since Cauna's review.

Phylogenetic Occurrence and Distribution

Since the early discovery of human Meissner corpuscles, these mechanoreceptors have been identified in some rodents, most marsupials and all primates (Munger and Ide, 1988). They have the same general basic structure, but display various degrees of complexity and size in different species as well as some individual variability. Ide (1976) has described the presence of Meissner corpuscles in murine glabrous digital skin. "Meissner-like" corpuscles were confined to the vestibular mucosa and to the lateral eminent mucosa, and in the vermilion border

of the rat lip (Tachibana et al. 1987). In raccoons, Meissner corpuscles were seen as one of several types of sensory end-organs in the glabrous skin (Turnbull and Rasmusson, 1986). Brenowitz, et al. (1980) found these corpuscles in the forepaw skin of young opossums and they were also identified in the skin of the marmoset (Perkins, 1970).

Locations in the body occupied by Meissner corpuscles have been best elucidated in primates, especially man. These corpuscles have been found in greatest number within the glabrous dermatoglyphic (having papillary ridges and/or rete pegs, see Munger and Ide, 1988) skin of the digits (Darian-Smith, 1982). Other areas of the body in which they are known to be found are the occlusal surface of the eyelid (Munger and Halata, 1984), the penis in nonhuman primates (Malinovsky and Sommerova, 1977; Halata and Munger, 1986), the vermilion border and mucosa of the lip (Halata and Munger, 1983), the buccal mucosa of the oral cavity (Griffin, 1985), and the palatine mucous membrane (Watanabe and Konig, 1977). Meissner corpuscles also occur in various tumors, such as the Wagner-Meissner neurilemmoma (Kaiserling and Geerts, 1986), and in some neurofibromas (Schochet and Barrett, 1974).

Mature Structure

The structure of Meissner corpuscles in the various mammalian species in which it is found is essentially constant except for differences in size. Studies have been most frequent in rodents and primates.

The digital corpuscle of the mouse toe pad has been well-described by electron microscopy (Ide, 1976). (Refer to Figures 1-5 in the Results for pictures of the murine corpuscle.) The corpuscle has an ellipsoid shape with the long axis perpendicular to the skin's surface. The corpuscle measures approximately 30 micrometers long and approximately 20 micrometers wide. One to three myelinated nerve fibers enter the corpuscle at its lower pole. The corpuscle consists of lamellar cells, nerve fibers, a capsule, and interstitial material. The lamellar cell bodies are usually located in the lower position of the corpuscle, and there are stacks of cytoplasmic plates or lamellae surrounding each nerve fiber.

The capsule consists of one layer of thin, cytoplasmic extensions from capsular cells located in the lower portion of the corpuscle. The capsule does not extend to the distal end where epithelium directly abuts the apex of the corpuscle. The capsule appears to be continuous with the perineurial epithelium, resembling it in morphology and containing similar organelles. Numerous caveoli invaginate the plasmalemma of the capsular cells. Basal laminae invest each cell on both inner and outer surfaces.

Lamellar cells envelope each nerve fiber with thin cytoplasmic plates or lamellae. The cell bodies of the lamellar cells are usually found in the lower portion of the corpuscle. This cell is characterized by a basal lamina, caveoli on both sides of the cell, and numerous filaments in the cytoplasm. The lamellae may play an important role in function of mechanoreceptors, by possibly providing nutrition to the nerve fiber or transmitting mechanical distortions to the receptor. The

cytoplasmic plates of the lamellar cells are layered in a hemilamellar fashion, parallel to the skin's surface and parallel to the axis of the terminal axon.

Both myelinated and unmyelinated nerve fibers enter the corpuscle. When a myelinated nerve fiber enters the corpuscle, it loses its myelin sheath and becomes enveloped by lamellar cells. The nerve fiber then courses tortuously towards the corpuscle's distal end. It sends off branches which course parallel to the skin's surface and are enlarged as discoid terminals. Each axon terminal is packed with numerous mitochondria. Numerous axonal processes, often referred to as axonal microprocesses, project from each terminal enlargement and are believed to be responsible for detection of pressure transients (Munger and Ide, 1987). These axonal microprocesses project into the space or cleft between the hemilamellae. The axonal processes have many vesicles at their base and are filled with numerous filaments, but are lacking mitochondria. The preterminal axon profiles within corpuscles generally contain fewer mitochondria. Their circular profile has a central region mainly composed of neural filaments and microtubules. Terminals occasionally are seen to extend from a corpuscle into the basal region of the epidermis.

The interstitial connective tissue within corpuscles consists of fine basal lamina material and scattered collagen fibrils. No connective tissue cells are found. The space between lamellae and axon, as well as between lamellae, is occasionally interrupted by gap junctions (Yoshida, et al. 1989). The organelles found in lamellar cells are mitochondria, rough endoplasmic reticulum, free ribosomes, Golgi bodies,

centriole, vesicles, microfilaments, and microtubules. Numerous caveoli line the plasmalemma of the cell bodies and lamellar processes. The nucleus of each lamellar cell is euchromatic, except for a peripheral zone of condensed chromatin. One or two prominent nucleoli can be seen.

The distribution of intramembranous particles as studied by freeze-fracture has been reported by Ide et al. (1985). In murine Meissner corpuscles, intramembranous particles in the terminal axolemma and in lamellar cell plasmalemma have much higher density than those of non-terminal axons or Schwann cells in myelinated and unmyelinated fibers. Also, intramembranous particles in the terminal axolemma are larger than those in non-terminal axolemma except for the nodal axolemma. Yoshida, et al. (1989) have documented similar findings in freeze-substituted Meissner corpuscles. These membrane specializations may be involved in the process of transduction. Intramembranous particles might represent various kinds of ion channels for sodium, potassium and calcium, and membrane-associated enzymes such as sodium potassium ATPase (Deguchi, et al. 1977), as well as structurally integrated proteins within the plasma membrane.

Meissner corpuscles in primates are very similar to those described in the mouse. The corpuscles were first studied by electron microscopy by Pease and Pallie (1959). Numerous reports on the fine structure of Meissner corpuscles were conducted during the next decade (Cauna and Ross, 1960; Munger, 1971, 1973, 1975a, b; Hashimoto, 1973; Halata, 1975; Chouchkov, 1973a, b, 1978; Castano, 1974; Castano and Ventura, 1978, 1979; Castano et al. 1985). The corpuscle is usually

ovoid in shape with a long axis perpendicular to the skin's surface, measuring 30 to 100 micrometers in length. The axon innervating Meissner corpuscles is single in most primates, but in man there are two or more axons (Cauna, 1956, 1958). Their morphology and ultrastructure are identical to that described above for murine corpuscles, except for their larger size.

Development

Studies of the development of Meissner corpuscles are relatively few in number (Polacek and Halata, 1970; Munger, 1975a). Cauna (1953) described by light microscopy human digital Meissner development. He observed the close association of lamellar and epidermal cells during Meissner corpuscle formation. Development is, in part, intraepidermal and, in part, extraepidermal in the perinatal period. Naked axons consistently lacking a Schwann cell investment enter the epidermis and small branches course between epidermal cells. Munger (1973, 1975b) has described, by electron microscopy, the developmental sequence of Meissner corpuscles in the tongue and palatal regions of the Rhesus monkey. His account is similar to the developmental sequence described for murine Meissner corpuscles, except these events occur postnatally in mice (Halata, 1981).

In Ide's study (1977) of the development of the Meissner corpuscle in the mouse toe pad, he noted no evidence of corpuscle formation prior to birth. Ide observed that by 18 days of gestation, neurites are seen near the epidermis but intraepidermal neurites increases, some accompanying Schwann cells extend their

cytoplasmic processes, penetrating the basal lamina of the epidermis by one day after birth. Four days after birth, Schwann cells invade the epidermis further, extending many cytoplasmic processes which are intimately associated with the basal cells of the epidermis. These specialized Schwann cells, after contacting the epidermis, begin to develop cytoplasmic lamellae. Schwann cells and neurites interact closely throughout corpuscle development with the epidermal cells. By 20 to 25 days after birth, the corpuscles are cytologically mature and located in the apex of dermal papillae.

A histochemical study of lamellar cell development of Meissner corpuscles has been done (Ide, 1982c), confirming that lamellar cells are derived from Schwann cells as suggested by Munger (1971). The lamellar cell is a specialized form of the Schwann cell which still retains the embryonal characteristics for synthesizing nonspecific cholinesterase. The question now arises as to what the significance of nonspecific cholinesterase activity is in the developing Schwann cells. Since cholinesterase activity is located in various cells, (the droplet cells of the primitive streak, endodermal cells, primordial germ cells, elongating lens fibers, and migrating neural-crest cells), the cholinesterase activity is likely related to the phase of active movement of Schwann cells during development. Drews (1975) has postulated that cholinesterase activity during embryonal development might be involved in the regulation of the morphogenic movement of cells, on the basis of the observations that cholinesterase activity generally appears in the blastodermal cells as soon as any particular organ starts to differentiate.

Basal laminae of lamellar cells have been postulated to have a specific role in the differentiation of developing axons and Schwann cells into specialized axon terminals and lamellar cells, respectively. Ide (1986) treated murine digital pads by freezing and thawing to cause the cellular constituents of the corpuscles to degenerate, leaving in situ basal laminae in their original configuration. By fifteen to twenty-five days after treatment, the Meissner corpuscles almost completely regenerated from growing axons and Schwann cell processes which extended into the basal laminae loops. This function of basal laminae serving as scaffolds for orderly tissue replacement has been noted in other tissues (Vracko, 1974; Vracko and Benditt, 1972). Basal laminae from various tissues are known to induce cell differentiation during development (see Ide, 1986 for a review). Thus, it is probable that the basal laminae of lamellar cells in Meissner corpuscles contain substances which might be responsible for inducing the differentiation of developing lamellar cells and axon terminals.

The trophic dependence of Meissner corpuscles on their sensory innervation is typical of sensory end-organs. If their nerve supply is transected, the axons and lamellar cells degenerate (Dellon, 1976; Dellon et al. 1975; Ide, 1982a; Dellon and Munger, 1983). Regenerating axons can enter degenerated corpuscles, and in most cases, result in redifferentiation of lamellar cells, forming regenerated, mature corpuscles (Ide, 1982b) that function normally (Sanders and Zimmerman, 1986).

Histochemistry

Meissner corpuscles display active non-specific cholinesterase (Winklemann, 1960; Cauna, 1960; Ide and Saito, 1980b), alkaline phosphatase (Ide and Saito, 1980a), and carbonic anhydrase activity (Tohyama and Ide, 1987). The cholinesterase activity can be found in the interspaces between the lamellae and in the periaxonal spaces between the lamellae and axon (Ide and Saito, 1980b). No enzyme activity is present in the axon. The cholinesterase enzyme is synthesized by the lamellar cell, as shown by activity in the cisternae of the rough endoplasmic reticulum and nuclear envelope. The activity is abolished when the cells are killed by freezing (Ide, 1986). Intense ATPase and alkaline phosphatase activity have only been described to date in murine Meissner corpuscles (Ide and Saito, 1980a). These enzymes are localized on the plasma membranes of lamellar cells and axon terminals. Carbonic anhydrase activity, on the other hand, is present in the axons (Tohyama and Ide, 1987). The axons also contain S-100 protein, neurofilament proteins, and neuron-specific enolase (Iwanaga et al. 1982).

Calcitonin gene-related peptide has been localized in about 15 percent of the Meissner corpuscles of the rat glabrous skin (Ishida-Yamamoto, et al. 1988). This double immunofluorescent cytochemical investigation has revealed that almost all the calcitonin gene-related peptide nerve fibers in the Meissner corpuscles are immuno-reactive for substance P. Correlative immuno-electron microscopic analysis has demonstrated that the calcitonin gene-related peptide fibers in the Meissner corpuscles are unmyelinated. Throughout the passage through the Meissner

corpuscle, no synaptic contact between the calcitonin gene-related peptide fibers and other parts of the Meissner corpuscles can be found. These fibers by their immuno-reactive properties are believed to be related to nociception. The role of these in the Meissner corpuscles is not understood. It should be noted that nerve endings of the myelinated fibers that mediate the mechanical tactile stimuli always lacked immuno-reactivity for calcitonin gene-related peptide. These findings suggest that the calcitonin gene-related peptide fibers in the Meissner corpuscles do not participate in the perception or transmission of the mechanical tactile stimuli to the central nervous system. Further studies are needed to clarify the functions of these fibers in the Meissner corpuscles.

Dipeptidylpeptidase-IV activities in Meissner corpuscles of the Rhesus monkey has been shown (Dubovy, 1988a,b). The enzyme activity was found in fibroblast-like cells forming an incomplete capsule around the Meissner corpuscle. Distinct electron dense reaction product was consistently localized in the plasma membrane of the lamellar cells. The axolemma was devoid of reaction product. Dipeptidylpeptidase-IV is a serine axopeptidase which removes x-proline sequences from n-terminal endings or peptides or artificial substrates. The capability of dipeptidylpeptidase-IV to cleave substance P has been demonstrated biochemically. The presence of this enzyme activity as well as the occurrence of substance P containing nerve fibers in Meissner corpuscles suggests the possible functional involvement of the enzyme in the production of substance P fragments in these

corpuscles. This supports the idea that unmyelinated nerve fibers in Meissner corpuscles might mediate the sense of pain and not touch.

The role of the other enzymes localized in Meissner corpuscles is not understood. Many hypotheses have been proposed, for example, to the physiological significance of carbonic anhydrase activity. Carbonic anhydrase is thought to be concerned with controlling the volume of water in the central nervous system. In the peripheral nervous system carbonic anhydrase also could control the intracellular concentration of hydrogen and chloride in some neurons. Thus, the reactivity in the lamellae might play an important role in the control of the ionic environment of the axon terminals. Recently, abundant gap junctions were found between the inner core of lamellar cells in Pacinian corpuscles using freeze-fracture methods (Ide and Hayashi, 1987). Gap junctions in the inner lamellae would imply electrotonic coupling with each block of hemilamellae. The presence of carbonic anhydrase activity thus might be related to the control of the ionic environment of the central axon in the mechanoelectric transduction.

The uptake of horseradish peroxidase by sensory terminals of lamellated corpuscles in mouse foot pads has been demonstrated (Jirmanova and Zelena, 1980). Injected horseradish peroxidase diffused into intralamellar and adaxonal clefts and was taken up by sensory terminals, being incorporated into coated pits and coated vesicles formed by the axolemma. Traces further appeared in the axoplasm in smooth vesicles, multivesicular bodies, and vacuoles. A similar incorporation of horseradish peroxidase into vesicles, inclusion bodies, and vacuoles was observed

in the lamellae and perinuclear cytoplasm of lamellar cells. Vibratory stimulation considered to be adequate for this type of mechanoreceptor did not result in any perceptible increase in horseradish peroxidase uptake by sensory terminals. This shows that the vesicles are probably involved with endocytosis and not neurotransmission of stimuli. The function of endocytosis at this time is not clearly understood. It is postulated that this reflects a high turnover of the plasma membrane in terminals engaged in the transduction process. It is also possible that endocytosis at sensory terminals is not a mere retrieval of redundant plasma membrane. It may be a part of an intracellular feedback system, or by regulatory macromolecules taken up in the periphery and brought by retrograde transport to the perikaryon mediate information about conditions in distant terminals and/or their microenvironment.

The significance of ATPase is still uncertain. It is probably related to transport processes at the plasma membrane. The ATPase demonstrated in the lamellar cells and nerve terminal plasma membranes is a magnesium ATPase, not the sodium potassium ATPase which is associated with the active transport. These enzymes might be involved in the transport of nutrients from outside the corpuscle to the nerve terminal.

Structural Basis of Function

Meissner corpuscles are rapidly adapting mechanoreceptors with a maximal sensitivity to vibration near 40 hertz (Darian-Smith, 1982; Munger and Ide, 1988).

Munger, et al. (1983) have characterized this parameter with discrete Meissner corpuscles in primate glabrous skin. A dorsal root ganglion was surgically excised from two adult Rhesus monkeys causing a reduced population density of sensory receptors in the digits two weeks later. The decreased density of mechanoreceptors in the skin made it easier to determine indentation thresholds, adaptive properties and receptive field boundaries. After each rapidly adapting receptor field was identified, the skin at the area was marked with india ink and on an enlarged photograph of the skin. At the completion of the physiological study the animals were perfused with aldehyde fixative and individual receptor sites excised and serially sectioned and impregnated with silver. Each rapidly adapting unit site contained a single innervated Meissner corpuscle.

Verrillo (1968) has formulated the duplex model of vibratory mechanoreception. On the basis of his experiments determining vibrotactile thresholds as a function of a number of spatial and temporal parameters of the physical stimulus there appear to be two populations of mechanoreceptors. The Pacinian corpuscles are responsible for summating transduced vibration energy over time and space. The non-Pacinian receptors are not capable of summation. Thus, Pacinian corpuscles provide afferent input that allows discrimination between different vibration frequencies as demonstrated by a U-shaped curve for frequencies above 40 Hz when vibration thresholds are plotted as a function of vibration frequency. Non-Pacinian receptors (including Meissner corpuscles) provide afferent input that produces a relatively flat curve for lower frequencies if plotted the same

way. This means that Meissner corpuscles, and other non-Pacinian receptors, are the main population activated at low frequencies (25 and 40 Hz), allowing fine two point discrimination at the skin surface; as frequency increases, more and more Pacinian receptors are activated, providing discrimination between frequencies.

Solely on anatomical grounds, Cauna (1954) had suggested that Meissner corpuscles were ideally suited for the "non-Pacinian" function. They have relatively high thresholds, receptive fields of limited size and have been suggested to be involved in making fine spatial discriminations (Lindblom, 1965).

LaMotte and Srinivasan (1987) have shown that Meissner corpuscles are important for the tactile discrimination of shape. On the monkey finger pad, each corpuscle responds to vertical velocity at the most sensitive spot on its receptive field, together with a response to the rate of change in skin curvature. This permits discrimination about the sharpness of shapes. LaMotte and Whitehouse (1986), studying the finger pad of monkeys by evoked responses, showed that receptor activity is activated by lateral deformation of elevated regions of skin (papillary ridges). Perceptive specificity of mechanoreceptors in general is reviewed by Torebjork, et al. 1987).

The mechanism of mechanoelectric transduction is poorly understood and is currently an area of intense investigation. Pacinian corpuscles have traditionally served as the model of choice in the studies of the transducer mechanism. These corpuscles are very similar to Meissner corpuscles. They have the same lamellar arrangement of cytoplasmic processes arranged in hemilamellae around a central

axon with very similar ultrastructural relationships and features; however, the intracorpuscular axon is not tortuous but is comparatively straight. There also is a different type of compartmentalization involving outer and inner lamellae. These differences in structure, in some yet unknown way, account for the different response characteristics of these two receptors. For further information on the structure of Pacinian corpuscles, please see the review by Munger and Ide, 1988.

Structural aspects of Pacinian and Meissner corpuscles believed to be of functional significance have been well outlined Munger and Ide (1988). In a recent study of Pacinian corpuscles using freeze fracture (Ide and Hayashi, 1987), much more information was gained regarding the understanding of function correlated with structure. The inner core has numerous gap junctions between lamellae. The gap junctions are limited to each hemi-inner core, that is, between lamellae only in each half of the inner core. Since gap junctions are low resistance junctions between cells (Loewenstein, et al. 1978), it is logical to conclude that the lamellar halves are separately electrotonically coupled. Munger and Ide (1987) have proposed that the halves of the inner core could be a sink or a source for potassium necessary for the rapid adapting properties of the receptor. Ilyinsky, et al. (1976) have provided intriguing data suggesting a high concentration of potassium in Pacinian corpuscles. Potassium ions caused a lowering of firing thresholds, thus increasing receptor excitability.

In contrast, the inner layers of the outer lamellae are coupled with tight junctions, not gap junctions, as identified by freeze fracture preparations (Ide and

Hayashi, (1987). Tight junctions function to establish barriers and thus prevent ionic flow. It has been suggested that the outer core is an expanded perineurial compartment and the inner core is in an expanded endoneurial compartment. This cellular organization would restrict any flow of ions and current from the inner to the outer compartment.

Studies of freeze fractured preparations of axolemma have noted differences in the distributions of intramembranous particles in the x and y axes (Ide and Hayashi, 1987). The x axis faces lamellae that tightly abut the axolemma and the y axis has numerous axonal spines that project into the connective tissue compartment of the cleft. This structural difference between the x and y axes may be related to the directional sensitivity of Pacinian corpuscles. Since compression produces depolarization, rotation of the corpuscle 90 degrees results in hyperpolarization (Loewenstein, 1971; Ilyinsky, 1965). Vibrations transmitted to the axon could result in different strains at the level of the axolemma of the x and y phases. Transduction of mechanical stimuli may be focused to the axonal spines of the y axis due to restriction of current flow at the x axis. Further studies are needed to understand the functional significance of this anisotropy.

An analogy has been suggested which compares the mechanoelectric transduction phenomena in mechanoreceptors to that in the hair cells of the cochlea. It has been demonstrated that the human finger can, in fact, perceive sound waves when water is the propagating agent (Munger and Ide, 1987). The structural specializations outlined above in mechanoreceptors are analogous in many respects

to the hair cells of the cochlea. The suggestion has been made that the core of Pacinian corpuscles has all the characteristics of a magnetorestrictive or electrorestrictive mechanoelectric transducer or sonar detector (Munger and Ide, 1987). The two hemi-inner cores sandwich the axon between them exactly as the restrictive elements sandwich the active element of a sonar detector. The presence of gap junctions in the inner core would presumptively imply that each half is electrotonically coupled. Such a functional entrapment of the central axon would provide a mechanism for establishing in an ion gradient that could keep portions of the axonal membrane in a state of partial depolarization. This would be similar to that of the organ of Corti where the endocochlear potential partially depolarizes the organ of Corti. The endolymph has a high potassium concentration, just as the Pacinian corpuscle inner compartment fluid, which is responsible for causing partial depolarization. Future studies with microelectrodes are clearly needed in order to determine the ion characteristics of the various portions of the inner core. If the clefts are to be considered as possible sites of restricted or facilitated ion flow, what structure specializations would be involved? The axonal spines of the cleft, as noted by Munger and Ide (1987), as well as the profusion of spines at the extreme tip of the axon, as noted by Ide et al. (1988), are the most likely candidates as the site of mechanoelectric transduction. Their filamentous content is analogous to the filaments in stereocilia of hair cells in the auditory system. This suggests that both the stereocilia of hair cells and the axonal spines of cutaneous mechanoreceptors possess membrane components that would interact with a pressure wave to initiate

transduction. The cleft of Pacinian corpuscles as the site of ion flow, is more likely since movement of ions is unlikely through the x axis of the axon facing the numerous lamellae of the inner core. The y axis is exposed to the connective tissue compartment of the cleft of the inner core as is the extreme tip. The extreme tip is continuous with the cleft, and the axon on both locations has similar structural specializations. The axon is thus suspended in one ion environment along the y axis and the cleft exposed to an entirely different ion environment along the x axis. This arrangement would compare the Pacinian corpuscles to a electrorestrictive mechano-electric transducer. The presence of filaments in axonal spines has been noted previously. The intriguing possibility that the filaments could resemble the actin filaments of hair cells, as described by Saunders, et al. (1985), offers another parallel between the auditory and cutaneous systems.

In Meissner corpuscles, the extracellular cleft is not isolated from the extracellular fluid outside the corpuscle due to an incomplete capsule and no inner core. Therefore, it is doubtful whether the potassium concentration is as high as in Pacinian corpuscles. There is probably less partial depolarization, and consequently, a higher firing threshold.

While the above discussion suggests that a compressive wave is an adequate stimulus for many cutaneous mechanoreceptors, the events at a molecular level are not understood. The effects of compression producing an on-depolarizing and on-hyperpolarizing response should be referred to as compressional sensitivity. The effects of compression producing either hyper- or depolarizing responses, and

rotation of the corpuscle by 90 degrees, giving the opposite reaction, should be referred to as directional sensitivity. Both indicate asymmetry and, thus, anisotropy within the receptor. The molecular mechanism involved here may not need to be a single mechanism.

Another similarity between the stereocilia of auditory hair cells and mechanoreceptors is what has been illustrated as the "greater membrane" concept using fish vestibular stereovilli membranes (Neugebauer, 1986). The stereovillar membrane, along with the cytoskeleton and extracellular material which support and connect the stereovilli can be considered together as a morphological unit. The common theme of seeing this triad in many mechanoreceptors indicates the importance as part of the transducer mechanism. A membrane coupled to the extracytoplasmic environment by intimate contact to extracellular material, as well as coupled to the cytoplasmic compartment by a cytoskeleton, likely represents the physical pathway whereby waves of mechanical stimuli are efficiently transmitted to the axolemma, thus triggering physical, then electrochemical changes. The physical change that "triggers" the mechanical to electrical transduction is probably membrane tension which seems to be common to several cell types, such as myofibers, neurons and epithelia. These cells have ion channels in the plasmalemma which are sensitive to membrane tension. Sachs (1986, 1988) has reviewed biophysical studies which demonstrate the channel is mechanically in parallel with cytoskeleton actin. This tension sensitive channel coupled to cytoskeleton, as a transductional model, explains the kinetic and sensitivity features of the cochlear-vestibular system. This proposed

mechanism can be broadly applied to a variety of mechanico-electrical sensory systems and account for existing data.

Grigg (1986) reviewed biophysical studies and made conclusions similar to those of Sachs. In addition, he elaborated on the ionic processes resulting after stimuli, conducted through elastic and viscoelastic material, act on the transducer. Channels which are cation specific and open in response to membrane strain in muscle cells were presented as a model for a spike-initiating depolarization in mechanoreceptors. A few other investigators (Kukushkina et al. 1988) recently explored the transducer mechanism in lamellated sensory corpuscles and made similar conclusions.

II. Age-Changes Directly or Indirectly Affecting Meissner Corpuscle Function

It is well known that somesthetic sensitivity declines in aging humans (Dyck, et al. 1972; Kenshalo, 1977, 1986). Sensitivity decline is most pronounced in the distal end of the lower extremities, and is least pronounced in the face. This decline is mainly attributed to neuropathy in the peripheral nerves and atrophy of primary sensory neurons and end-organs. While not all the possible age-changes which might be causative factors for sensory decrement have been thoroughly analyzed, much has become evident. Ultrastructural age-changes of somesthetic sense organs have been studied in murine Pacinian corpuscles (Nava, 1988) and Merkel domes from vitamin A deficient rats (Baumann et al., 1986). Light microscopic age changes of Meissner and Pacinian corpuscles have been described, as well as light and electron (see

Discussion) microscopic changes of primary sensory neurons. Changes in aging skin may also indirectly affect mechanoreceptor response (Pubols, 1988). Other factors which have rarely or never been studied include age-changes in the sensory processing pathways of the central nervous system, the efferent modulation of mechanoreceptor activity (Johnson, 1988), and the biochemical mileu around and within all components of the system.

Structural Changes Within Meissner Corpuscles

Meissner corpuscles are known to undergo changes in number and morphology with advancing age. Several studies have demonstrated a loss of corpuscle numbers per area in the dermatoglyphic, glabrous skin of aging humans (Perez, 1931; Ronge, 1943; Dickens, et al. 1963; Cauna, 1965; Bolton, et al. 1966; Ridley, 1968, 69; Hunter, et al. 1969; Bruce, 1980; Schimrigk and Ruttinger, 1980). The remaining corpuscles become elongated and coiled, separated from the epidermis, and less uniformly distributed within the skin (Cauna, 1956, 1965; Ridley, 1969; Bolton, et al. 1966; Bruce, 1980; Schirmigk and Ruttinger, 1980). Axon terminals are lost in the proximal half of corpuscles. Those terminals remaining contain a network of neurofibrils and then at advanced age, become irregular, winding and attenuated (Cauna, 1956, 1965). Recent research utilizing morphometric techniques (Schimrigk and Ruttinger, 1980; Matsuoka, et al. 1983) has shown that corpuscles increase in length and cross-sectional area until man reaches

middle age, but thereafter decrease in size until many atrophy completely at advanced age.

In a series of investigations focused on describing murine sensory end-organs after silver-impregnation, age-related changes were seen to be similar to those depicted in humans (for a review of age-changes in human sensory end-organs, see Meisami, 1988). In forepaw digital pads of mice (Mathewson and Nava, 1985), Meissner corpuscle numbers and axon numbers per corpuscle were found to decrease with age. Qualitative and quantitative morphologic changes were also observed, such as increased axonal branching, tortuosity, varicosity and diameter until middle age, with attenuation and disarrangement of axons at more advanced age. These findings are supported by similar age-related changes found in a study of murine Meissner corpuscles in diabetic and age-matched non-diabetic littermates (Ras and Nava, 1986).

Similar Changes in Non-Meissner Sensory Endings

Analogous age-changes were also observed in the crural Pacinian corpuscles of the mouse (Nava, 1988), showing a "shift" to more complex axonal morphology at advanced age, largely due to increased tortuosity and branching of the single innervating nerve fiber. (Electron microscopic changes are presented in the Discussion). Corpuscular nerve endings in the rat snout also gain tortuosity and branching with age (Macintosh and Sinclair, 1978). Another study of the aging

mouse has shown a significant loss of somatosensory and gustatory nerve fibers innervating the fungiform papillae (Nava, et al., 1983).

Changes in Peripheral Nerves

A decrease in the number of primary sensory neurons may occur with advancing age. This would account, at least partially, for the loss of mechanoreceptors with age and would undoubtedly affect cutaneous sensitivity. Corbin and Gardner (1937) reported a 32 percent reduction in the number of human spinal ganglion cells. A decrease of sensory neurons may be suggested by age-related decreases of peripheral nerve fibers. In the anterior tibial nerve in humans, Swallow (1966) noticed a decrease with age in the number of large-diameter axons. Samorajski (1974) reported an age-related decrease in the total number of myelinated posterior tibial nerve fibers in mice with a fairly uniform loss across all fiber diameters. Ochoa and Mair (1969) observed an age-related degeneration of myelinated sural nerve fibers in humans. However, rat sciatic, tibial, and medial plantar nerve fibers did not decrease in number with age (Birren and Wall, 1956; Sharma, et al. 1980). There is conflicting evidence regarding the cause of reduction in the number of human unmyelinated fibers with age (Behse, et al. 1975).

Changes in the diameter of peripheral nerve fibers would affect conduction velocity of sensory impulses generated by mechanoreceptors. Changes of the diameter of the peripheral nerve fibers in general could suggest diameter changes of the sensory nerve fibers contained in the peripheral nerve. In rats, Sharma, et al.

(1980) found an initial rapid increase in nerve fiber diameter, followed by a more gradual increase which ceased after approximately nine months of age in tibial nerve, but continued for longer in the medial plantar nerve. Fiber size distribution remained unimodal throughout aging. Maximal and average fiber diameter became reduced by age 24 months. The above data suggests that conduction velocities increase until middle age, then decrease thereafter.

Studies have been done of age-changes of conduction velocity. The maximum velocity of human medial plantar nerve fibers for 50 to 69-year old humans dropped by 10 meters per second as compared to 10 to 29-year olds (Sommer, 1941). Wagman and Lesse (1952) found a decrease in velocity in human ulnar nerve fibers of about 7 meters per second between the third and seventh decade. Norris, et al. (1953) reported the decrease of velocity in human ulnar nerve fibers of about 10 meters per second from the ages of 30-39 to ages 80-89. Birren and Wall (1956), however, report the most significant change in conduction velocity in rat sciatic nerve fibers. If motor impulse conduction velocity changes with age, probably sensory impulse velocity does likewise. Spencer and Ochoa (1981) state that there is a documented decrease of impulse conduction with age in sensory nerve fibers, and conclude that this is probably one reason for reported age-related decreases of cutaneous sensation.

In humans, cytons of primary sensory neurons are known to exhibit age-related structural changes (Hess, 1955). With age, proximal and distal axons extending from these cytons and spinal ganglia are lost and eventually replaced by

connective tissue (Spencer and Ochoa, 1981). Abnormalities and axonal degeneration in tibial and plantar nerves of the rat have been reported to increase with age (Sharma, et al. 1980). Measurable size of structural decay in peripheral nerve fibers have been defined by many others (Spencer and Ochoa, 1981). (See Discussion for electron microscopic changes.)

Changes in The Physical Properties of the Skin

One possible factor affecting cutaneous sensitivity with age is altered mechanical properties of elderly skin. If the physical properties of skin change with age in such a way as to alter the conduction of mechanical stimuli from the skin surface to the mechanoreceptors in deeper tissues, then this would be an important factor affecting age-changes of cutaneous sensitivity. Histological studies show that there are decreased amounts of elastin and collagen in elderly skin. What remains appears increasingly stable. Consistently, young rat skin has greater tensile strength than older rat skin. In general, the mechanical properties of skin deteriorate with age, as shown by the loss of elastic recovery and prolonged recovery from indentation. Overall, the aged dermis appears to become an increasingly rigid tissue, less able than young skin to undergo biochemical or mechanical change in response to stress (for review see Kenshalo, 1986).

Structurally the aged epidermis becomes thinner, the cornified cells become less adherent to one another, and there is flattening of the dermal epidermal interface. The dermis becomes atrophied and is relatively acellular and avascular.

Dermocollagen, elastin, and glycosaminoglycans are altered (for a review, see Fenske and Lober, 1986).

The mechanical properties of skin were studied in rats of different ages by Baumann, et al. (1986). They showed a linear decrease in skin compliance between young and old rats. No other studies have been seen in which comparisons have been made of the impedances or compliances of young and elderly skin. A decrease in compliance and thus an increase in impedance would affect the conduction and nature of pressure waves transmitted between stimuli at the skin surface and mechanoreceptors in deeper tissues. What effect this may have on cutaneous sensitivity is not known.

III. Purpose of the Current Investigation

To date, only one study (Nava, 1988) has utilized electron microscopy to analyze age-related structural changes in a cutaneous sensory end-organ. The present effort is the second study to use electron microscopy.

Structural changes found in Meissner corpuscles will assist in understanding age-associated distal axonopathy (Vlassara, 1988), and will help efforts to understand the apparatus producing mechano-electric transduction when subsequent electrophysiological correlation is attempted. In the present investigation, therefore, murine Meissner corpuscles were analyzed for morphometric and ultrastructural age-changes which likely affect tactile sensitivity. This work follows a preliminary study utilizing silver-impregnation (Mathewson and Nava, 1985).

MATERIALS AND METHODS

Animal and Tissue Preparation

Swiss Webster female albino mice from Simonson Laboratory were maintained at 24 degrees C with four to seven mice per shoebox cage. (A "shoebox cage" is a standard stainless steel box (33x18x15 cm) used to house small animals. It is covered with a perforated lid containing a suspended food container and water bottle.) Fresh bedding (wood shavings, 5 cm deep) was provided twice weekly. Standardized nondeficient diet (Rodent Laboratory Chow 5001) and water were given ad libitum. The daily light/dark ratio was 12/12 hours. Animals with gross pathological abnormalities were not studied. Six mice at ages of 1.5, 3, 6, 9, 12, 15, 18, 21 months each and three mice at 24 and 26 months each, were anesthetized with Nembutal (30-50 mg sodium pentobarbital per kg, i.p.) and perfused by cardiac intra-aortic cannulation with a 0.05 M cacodylate buffered solution of 3% glutaraldehyde, 1.5% paraformaldehyde and 1.5% acrolein. Digital pads were immediately excised from the forepaws and immersed in a 0.15 M cacodylate buffered rinse, followed by a postfix of 0.3 M cacodylate buffered solution of 1% osmium tetroxide for 4 hours at 4 C. Tissues were dehydrated in acetone and embedded in Epon 812.

For descriptive simplicity, the life span of this mouse strain (mean life span - 16.35 mo., maximum life span = 28 mo.) was divided into three stages: young age (1.5, 3, 6 mo.), middle age (9, 12, 15 mo.) and old age (18, 21, 24, 26 mo.).

Light Microscopic Analysis

To determine whether changes in corpuscle length, cross-sectional area, and volume occur with age, the following procedure was performed. One Epon-embedded digital pad was randomly selected from each mouse. Serial cross-sections (2 μm) were cut from the apex of each digital pad and stained with toluidine blue (Fig. 1). Enough sections were obtained so that at least four Meissner corpuscles per digital pad were serially cross-sectioned through their entire length. Quantitative analysis was done using a Zeiss Videoplan computer. Cross-sections of the digital pads were projected onto the planimeter using camera lucida at a magnification of x3040, thus allowing the cross-sectional area at each successive level of a corpuscle to be measured. The volume of each of the corpuscles in each digital pad were calculated. Mean average values for length, cross-sectional area, and maximal cross-sectional area were also calculated for corpuscles in each pad. Corpuscle size variation in each pad was determined as well.

Light micrographs of the serial cross-sections, as well as thick sections obtained for corpuscle localization during electron microscopy, were examined qualitatively for age-changes of the corpuscle-epidermal junction, corpuscle shape, and corpuscle size variation.

Electron Microscopic Analysis

At least two Epon embedded digital pads were selected randomly from each mouse at all sampled ages. Thick sections (2 μm) stained with toluidine blue were

utilized to locate transverse and longitudinal sections of the approximate center of corpuscles. Thin (silver) sections were then cut and collected on Formvar coated (1x2 mm oval-slit, copper) grids and stained with aqueous solutions of uranyl acetate and lead citrate (Sato, 1967). Some sections from other planes and levels of the corpuscles were also obtained. Corpuscles examined on a Siemens Elmiskop 1A electron microscope were recorded on 6.5 x 9 cm plates at magnifications of x2967, x5394 and x9391, then enlarged three times with final magnifications of x9000, x16,000 and x28,000. These were analyzed qualitatively for age-changes.

RESULTS

Corpuscle Structure at Young Age

Murine Meissner corpuscles, located at the apex of dermal papillae, are oval bodies measuring approximately 10-30 cm in diameter when "cytologically mature" at age 20-25 days (Ide, 1976, 1977). They consist of four elements: axons, lamellar cells, capsular cells, and interstitial material (Fig. 2 and 3).

Usually one or two axons, 4-5 μm in diameter, entered the proximal end of a corpuscle while losing their myelin sheaths and coursed tortuously (like "ribbon candy", Munger and Ide, 1988) toward the distal end while invested by lamellar cell processes. In addition, thinner unmyelinated axons occasionally entered a corpuscle before continuing into the epidermis, but had minimal association with the lamellae. Axons often ramified at points distal to the their entrance, each branch usually oriented parallel to the skin surface, terminating as a flat, ellipsoid enlargement containing numerous mitochondria and vesicles. Bilateral stacks of lamellae were also arranged parallel to the skin surface, as was the cleft between them. Small axonal processes extended from the terminal enlargements into the cleft (Fig. 3). These axonal processes had many vesicles at their base and were filled with numerous filaments, but lacked mitochondria. Preterminal axon profiles within corpuscles generally contained fewer mitochondria and vesicular profiles, with a central region mainly composed of neurofilaments and microtubules. Terminals

occasionally were seen to extend from a corpuscle into the basal region of the epidermis (Fig. 2).

Lamellar cell bodies were typically positioned proximally within a corpuscle, adjacent to the capsule. Basal lamina completely enveloped each lamellar cell body, but the lamellar processes were focally devoid of basal lamina (Fig. 3). Mitochondria, rough endoplasmic reticulum, free ribosomes, Golgi bodies, centrioles, vesicles, microfilaments and microtubules filled the cytoplasm. Numerous caveoli lined the plasmalemma of the cell bodies and processes. The nucleus of each lamellar cell was euchromatic, except for a peripheral zone of condensed chromatin. One or two prominent nucleoli were seen.

The capsule extended from the perineurium (Fig. 2) as thin epithelial cells invested by basal lamina. The plasmalemma of the capsule cells was invaginated with numerous caveoli. The capsule did not enclose the distal end of the corpuscle where it abutted the basal lamina of the epidermis.

The interstitial connective tissue within corpuscles consisted of fine basal lamina material and scattered collagen fibrils. No connective tissue cells were found. The space between lamellae and axon, as well as between lamellae, (Fig. 3) was occasionally interrupted by gap junctions (Ide, et al., 1985; Yoshida, et al., 1989).

Light Microscopic Age-changes

Meissner corpuscles looked larger and more complex at middle age than young age, but at old age, became smaller and lobulated, losing their more regular

organization (Figs. 4a-i and 5a,b). No age-changes in the corpuscle-epithelial junction were seen. Variation of corpuscle size appeared constant with age.

Morphometric data revealed significant changes of corpuscle length ($p<.05$), cross-area ($p<.01$), maximal cross-sectional area ($p<.05$), and volume ($p<.01$) occurring as inverse parabolic functions of age (Figs. 6a-d). Variation of corpuscle size did not change significantly with age (ANOVA: d.f.=7,34; $p<.1$).

Electron Microscopic Age-changes

Similar findings as noted above were observed by looking at low magnification electron micrographs of corpuscles (Figs. 7-10). Ultrastructural age-changes were evident as well.

No intraepidermal terminals extending from corpuscles (Fig. 2) were found after 1.5 months of age. At 3 months, size and structural complexity of some corpuscles were greatly increased (compare Figs. 2 and 7). More axon profiles surrounded by greater numbers of closely apposed lamellae were seen at middle age. By 9 months, most of the corpuscles demonstrated this complexity (Fig. 8). With advancing age, an increase in extracellular material was noticeable (compare Figs. 7-10). The fine basal lamina material and collagen fibrils became more plentiful between adjacent lamellae as well as next to lamellar cell bodies and capsular cells (Figs. 11 and 12). This more abundant interstitial material appeared to be basal lamina duplication in some regions.

Some corpuscles, mostly seen at middle age (Fig. 13), contained several irregular axon profiles, each enclosed by one to three lamellae with few caveoli. Irregularly arranged mitochondria, neurofilaments, microtubules and vesicles were observed inside axons (Figs. 13 and 14). Normally, mitochondria are peripherally arranged around centrally located neurofilaments and microtubules, except in the terminal enlargements where mitochondria tend to occupy most of the volume. The mitochondria were pleomorphic in shape, ranging from dilated, multivesicular bodies to small, dense bodies. Basal lamina duplication was evident between the cytoplasmic processes.

With more advancing age, corpuscles became disorganized, losing their regular structural pattern. The typical orientation of flat ellipsoid axon terminals parallel to the skin surface, invested by similarly oriented stacks of hemilamellae, was less commonly seen. Consequently, proportionally less of the neural and lamellar components of each corpuscle were horizontally arranged like "ribbon candy".

At old age, atrophic corpuscles were often identified. Lipofuscin was found in lamellar cells at 24 and 26 months of age (Fig. 14). In many axons, degenerative mitochondrial changes were apparent (Figs. 12 and 15). Corpuscles had abundant extracellular material but few lamellae around irregular or absent axons (Fig. 16). Lamellar processes often appeared attenuated with few caveoli (Fig. 17). The axon profiles, when present, usually were attenuated and lacked axonal processes (Fig. 17). Obvious basal lamina duplication occupied the vacated space once occupied by

lamellae and axons (Fig. 18). Regression of the perineurial epithelium of the capsule was also evident (Fig. 10 & 16).

DISCUSSION

Summary of Findings

Age-related structural changes of murine Meissner corpuscles have been observed in the present investigation and are summarized below. No intraepidermal nerve fibers continued from corpuscles after 1.5 months. Corpuscles typically became larger and more complex until middle age, then became disorganized and lobulated at old age. Corpuscular axons often became attenuated at advanced age with the axoplasm becoming electron-dense and the organelles losing their usual location - mitochondria are normally peripherally located around centrally placed neurofilaments and microtubules. Axonal processes were rare. Mitochondria became electron-dense, or dilated and reticulated. Lamellae became attenuated, fewer in number and electron-dense, with fewer caveoli. Lipofuscin accumulation was seen in lamellar cells only at very advanced ages. Fine basal lamina material and collagen fibrils increased with age, as well as basal lamina duplication. Regression of the perineurial epithelium of the capsule was also seen with advancing age.

Intraepidermal Terminals

In the first detailed account of the structure of mouse digital corpuscles (Ide, 1976), axon terminals were described to typically continue from the corpuscle in to the basal layer of the epidermis. Intraepidermal axons extending from corpuscles were seen occasionally at 1.5 months in the present study but were not seen in older animals. Using silver impregnation (Mathewson and Nava, 1985), which does not

produce as clear a demarcation between papillary dermis and epidermis as seen with electron microscopy, intraepithelial axons were rarely seen to extend from corpuscles after 1.5 months of age. It should be noted that Ide used animals that were less than 1.5 months of age for studying the structure of these corpuscles. In his study of developing corpuscles Ide (1977), found intraepithelial axons consistently during corpuscle development. We can conclude that these intraepidermal axons extending from corpuscles are not a component of the "mature" corpuscle but perhaps have an inductive role in corpuscle development. These should not be confused with the smaller non-myelinated fibers often seen to enter murine (Ide, 1976) and human (Cauna and Ross, 1960) corpuscles, then to continue deep into the epidermis as "free nerve endings" (Cauna, 1980; Novotny and Gommert-Novotny, 1988).

Morphological Age-Changes

Age-changes of murine corpuscle size were quantitated in this study in order to compare results with prior morphometric studies on human corpuscles (Schimrigk and Ruttinger, 1980; Matsuoka, et al., 1983). Comparison of the data indicate that the length and cross-sectional area of murine and human corpuscles increase until middle age, then with more advanced age, both parameters decrease until many corpuscles atrophy completely at old age. The increasing size of these corpuscles until middle age of both species may simply be a consequence of nonspecific growth of tissue which is mainly due to continuing formation and accumulation of collagen. Electron micrographs of aging corpuscles in the present study clearly showed more

collagen between the cellular elements. But growth of the axons also contributed to the increased corpuscle size. Corpuscles appeared to gain more horizontally arranged terminals, associated lamellae, and connective tissue. By increasing the amount of neural surface for mechano-electric transduction, this was thought to compensate for a loss of corpuscles and innervating axon numbers per corpuscle (Mathewson and Nava, 1985).

A few of these age-changes in murine Meissner corpuscles resemble findings first described by Cauna (1956, 1965) in human corpuscles of the aging skin, which he studied using light microscopy. He too observed that corpuscles become elongated, lobulated and disorganized with advancing age. However, no evidence was presently found for "loosening" or separation of the corpuscle-epidermal junction which he observed in humans. Aging murine corpuscles retain very close apposition to the basal layer of the epidermis at the apex of the dermal papilla.

Ultrastructural Age-Changes

Similar age-changes to those described in the present study were described in an electron microscopic analysis of murine Pacinian corpuscles (Nava, 1988), which essentially contain the same structural components as Meissner corpuscles (Munger and Ide, 1988). In aging Pacinian corpuscles, degenerative mitochondrial changes ranged from swollen, vacuolated and laminated to pleomorphic-appearing structures. The usual peripheral subaxolemmal position of mitochondria seen at young age changed to a more random arrangement of mitochondria within the axon.

Large, laminated, circular myelin-like bodies and lipofuscin accumulation were noted in axons of older mice. Axon profiles lost their ellipsoidal shape, became smaller, and were often absent at advanced age. Multiple axon profiles were seen in older Pacinian corpuscles which is evidence of increased branching of the single innervating nerve fiber. Basal lamina duplication was observed and extracellular material increased with age. At 24 months, lipofuscin was found in the lamellar cells, and lamellar cell processes had fewer caveoli.

While no other ultrastructural studies of aging somesthetic sensory end-organs have been performed to my knowledge, the primary sensory neuron innervating these sensory receptors has been examined at more proximal locations. Four types of neuropathologic processes have been encountered, as described by Vlassara (1988): lipofuscin accumulation, demyelination and re-myelination, low grade distal axonopathy, and neuronal loss. These processes seem to affect aging murine Meissner and Pacinian corpuscles, as described above. Their effect more proximally on the aging primary sensory neurons is similar (for reviews, see Spencer and Ochoa, 1981; Johnson, 1985). Lipofuscin accumulates in the cytons but commences at earlier ages. There are no reports of lipofuscin accumulation in more distal parts of these neurons, except in Pacinian corpuscles, as noted above. Age-related distal axonopathy in peripheral nerves is characterized by an intra-axonal accumulation of mitochondria, dense membranous bodies, glycogenosomes and polyglucosan bodies, and the presence of Hirano bodies within adaxonal Schwann cell cytoplasm.

Excessive endoneurial connective tissue and empty tubes of basal lamina remain after nerve fiber loss.

Lipofuscin accumulation is the most consistently described age-change in nervous tissue. Ultrastructurally, lipofuscin has four components (Sohal and Wolfe, 1986): a finely granular electron-dense material, coarse dense granules, osmiophilic lamellae and lipid-like vacuoles. These membrane-bounded organelles vary between 0.5-3 μm in diameter. The coarse granules are composed mainly of oxidized lipids which are largely insoluble in the usual lipid solvents (Johnson, 1985). Lipofuscin likely represents non-degradable waste products derived from partially broken down membranes and other cell components. The general consensus is that it belongs to the category of secondary lysosomes, and many believe that densification and vacuolation of mitochondria are involved in its formation (Wisniewski and Wen, 1988). Lipofuscin accumulation is thought to result from an irreversible build-up of membrane peroxidation products which the post-mitotic cell cannot discard. The peroxidation is widely believed to involve oxygen-derived free radicals (Sohal and Wolfe, 1986). It is controversial whether lipofuscin has a detrimental effect on cellular function, results from a deterioration of function, or is harmless, inert material. It has been shown that as lipofuscin collects in the cell body, the Nissl substance becomes dispersed and the total cytoplasmic content of RNA is reduced (Mann and Yates, 1974). If an accurate index of cell fitness lies with an estimate of cytoplasmic RNA content (the potential for protein synthesis), as the latter cited

author's believe it does, then increased lipofuscin results in a reduction of protein synthesis, inevitably leading to cell atrophy and death.

Mitochondria degeneration seen in the present study was similarly described in the aging optic nerves of mice (Johnson, 1978) and Merkel domes of vitamin A deficient rats (Baumann, et al., 1986). According to the mitochondrial mutation theory of aging, during maturation of the organism an increasing number of mitochondria lose the ability to divide as a consequence of mitochondrial DNA injury, and thus become susceptible to progressive membrane damage secondary to lipid peroxidation and cross-linking (Miguel, et al., 1983). This would explain the increasing number of degenerating mitochondria seen with advancing age. The fact that vitamin A deficiency accelerates mitochondrial degeneration (Baumann, et al., 1986) is consistent with this hypothesis since it is a well-known antioxidant preventing peroxidation.

At the distal end of the peripheral axon of primary sensory neurons, where the sensory end-organs are located, age-related axonopathy and supporting cell changes may be a consequence of age-changes in axonal transport (McMartin, 1983; Brunetti, et al., 1987; and Goemaere- Vanneste, et al., 1988). Most axonal macromolecules are synthesized only in the soma. Maintenance of terminals is therefore highly dependent on transport along the axon. This is well demonstrated by the degeneration of axons and end-organs after nerve transection. In degenerating murine Meissner corpuscles after nerve transection, Ide (1982a) described changes that are similar to the age-changes observed in the present study.

After denervation, he reported that the axoplasm of corpuscle terminals became electron-dense, mitochondria became swollen, and axonal processes were lost. Dense bodies and myelin figures increased in number. Lamellar cell processes became thinner and more electron-dense, with fewer caveoli along the plasmalemma. This similarity between denervation- and age-related neuropathy can be expected if both processes are considered to result from a decline in axonal transport.

Probable Functional Consequences

Declining cutaneous sensitivity to vibration and touch with age, has been mainly attributed to loss of sensory end-organs (Thornbury and Mistretta, 1981) and primary sensory neurons, as well as neuropathy in peripheral nerves. The question of whether the end-organs decline in sensitivity as a result of age-related structural changes has not been the focus of investigation. The present findings in aging murine Meissner corpuscles demonstrate changes, which based on current theory of mechanoreceptor structure-function correlation, (Munger and Ide, 1987; Bolanowski, 1988) should affect cutaneous sensation. Age-changes that resemble denervation changes, as described above, should affect sensitivity, especially since denervation-like changes were seen in a large proportion of the corpuscle population. Corpuscle age-changes that include a loss of axonal processes seem very significant functionally because axonal processes have been implicated for over a decade as a key component of the proposed apparatus producing mechano-electric transduction in

Pacinian corpuscles and other mechanoreceptors. The axonal processes in Meissner corpuscles are very similar, if not identical.

A functional decrement would seem to result from aging Meissner corpuscles that lose the regular arrangement of flat horizontal plates of terminal axons invested by similarly oriented stacks of hemilamellae. The resulting proportional loss of neural surface oriented parallel to the skin surface may cause reduction of the anisotropic response to stimuli, a characteristic demonstrated by Pacinian corpuscles shown to respond with depolarization only to compression directed along the minor axis (Nishi and Sato, 1968; Lowenstein, 1971).

An increased amount of collagenous connective tissue with age may have a significant effect on the physical transmission of pressure waves to the transducer. Even though no loosening of the corpuscle-epithelium interface occurs with age, increasing impedence between the corpuscle and epithelium may be a factor in decreasing sensitivity with age.

Future Efforts

In the future efforts focused on studying aging sensory systems, end-organ studies using electron microscopy rather than studying the already well-documented loss of end-organ and sensory neuron numbers. Neurochemical research in aging might provide information likely to affect mechano-electric transduction, such as changes in membrane properties. Additional work might also clarify the functional significance of age-related structural changes by employing electrophysiological

techniques such as intraneural micrography on afferents located closely proximal to their respective terminal sense organs.

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FIGURE LEGENDS

Figure 1: Photomicrograph of a digital pad at age 1.5 months sectioned horizontally, parallel to the skin surface, showing three corpuscles (asterisks) in dermal papillae. x125.

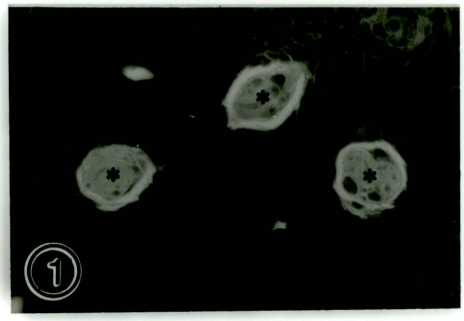
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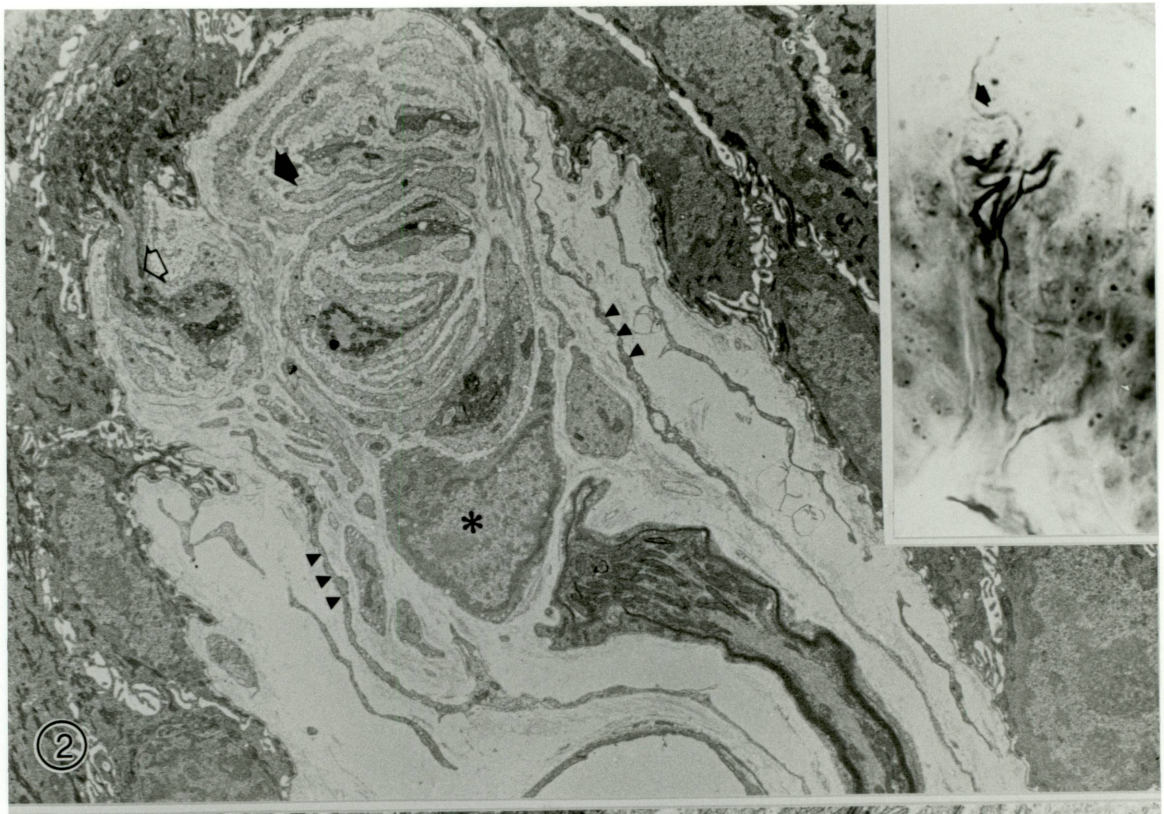
Figure 2: Electron micrograph of a corpuscle at age 1.5 months. An axon is seen as it enters the proximal end of a corpuscle, where it loses its myelin sheath, and courses tortuously. It exits the distal end to enter the epidermis, terminating as an enlargement densely filled with mitochondria (open arrow). Two to three stacks of hemilamellae enclose the axon, with a cleft oriented roughly parallel to the skin surface (closed arrow). A lamellar cell body is at the proximal end of the corpuscle (asterisk). The capsule is seen to be continuous with the perineurium (triangles). x5600. Inset: Photomicrograph of a silver-impregnated 70 μm thick section showing axons as they course tortuously through a corpuscle at age 1.5 months. Note the axon that continues into the epidermis (arrow). (This micrograph was published in J. Comp. Neurol. 1985 and is reprinted with permission of the publisher.) x800.

Figure 3: Electron micrograph of a portion of a corpuscle at age 1.5 months showing two axon profiles (A) and corresponding lamellar investments (L). Note the axonal processes extending into the cleft between hemilamellae (large arrows). Gap junctions between adjacent lamellae occur at random locations (small arrows). Basal lamina material and small collagen fibrils line the extracellular spaces. x19,000.



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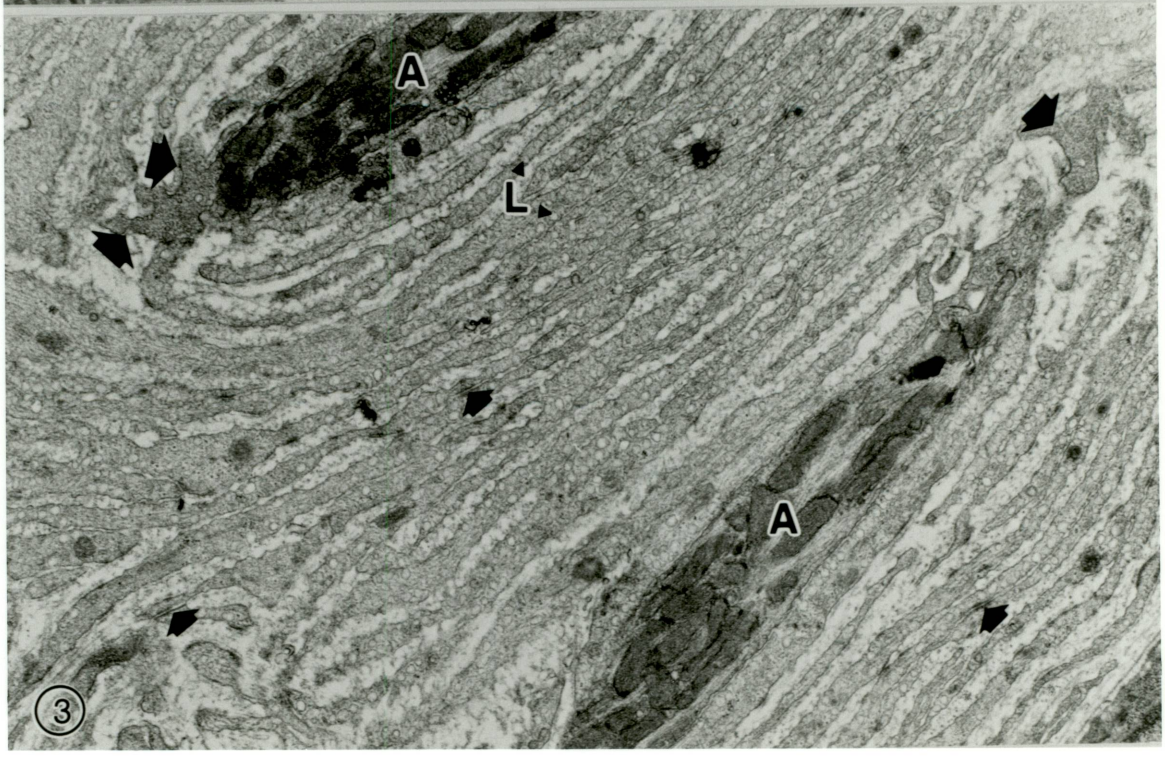
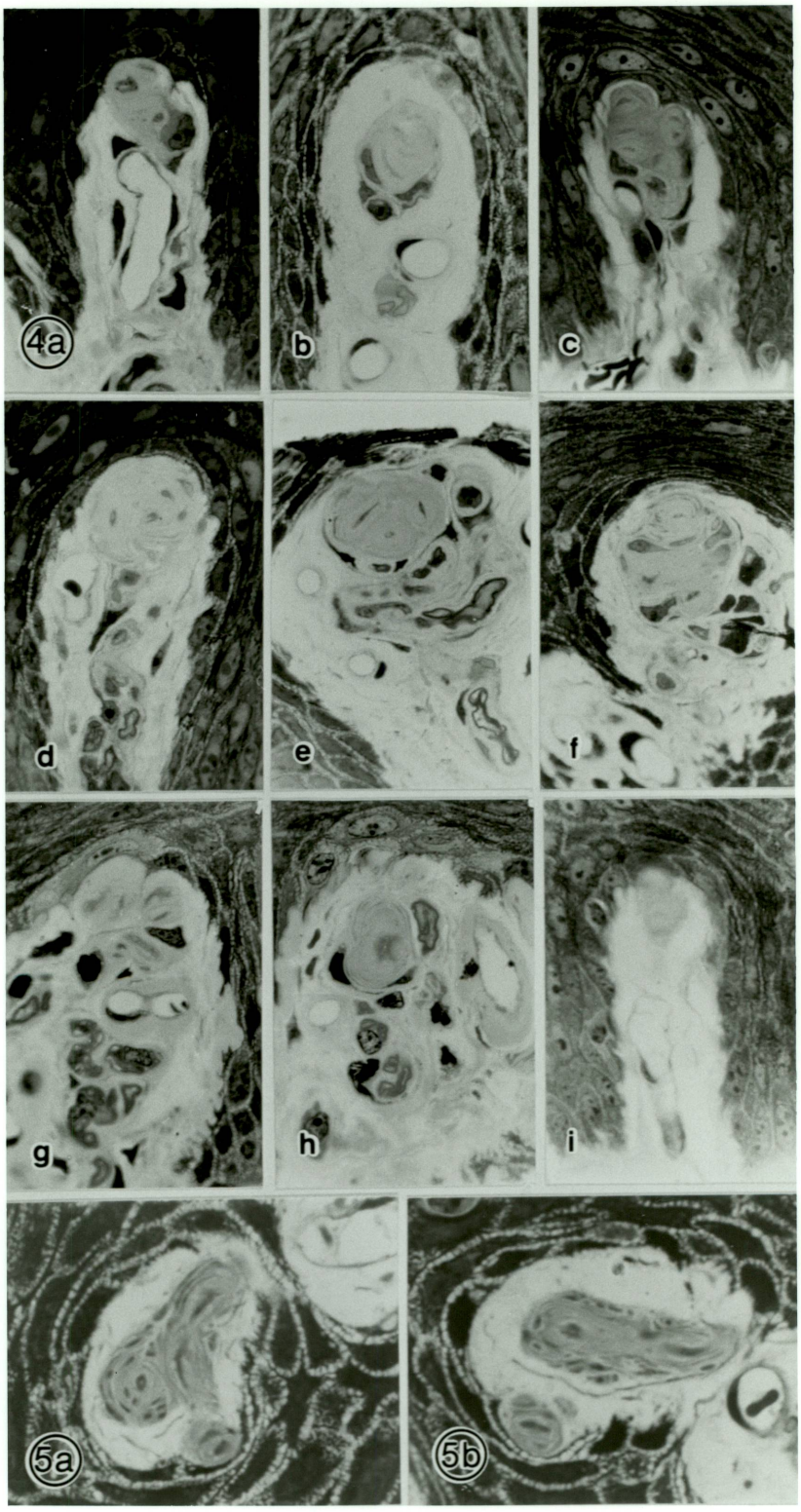


③

Figures 4a-i: Photomicrographs of 2 μm thick, Epon-embedded sections of mouse digital pads stained with toluidine blue showing Meissner corpuscles at the ices of dermal papillae. Ages represented are 1.5, 3, 9, 12, 15, 18, 21, 24 and 26 months, respectively. From young to middle age, the corpuscles become larger and more complex. At old age the corpuscles become smaller and lobulated. x200.

Figures 5a & 5b: Photomicrographs showing a corpuscle from a 24 month old mouse at two successive planes of 2 μm sections. Compare with Figure 1 and note the lobulated, disorganized character. x300.


4a
b
c
d
e
f
g
h
i
5a
5b

Figures 6a-d: Graphs showing the relationship with age for corpuscle length, cross-sectional area, maximal cross-sectional area and volume, respectively. Data points represent mean values for each mouse. All data significantly correlates to an inverse parabolic function which is a positive curvilinear regression from young to middle age and negative curvilinear regression from middle to old age. (For $p<.05$ at d.f.=2,31, the quadratic regression coefficient (r_q) = .349; for $p<.01$ at d.f. - 2,31, r_q = .449.)

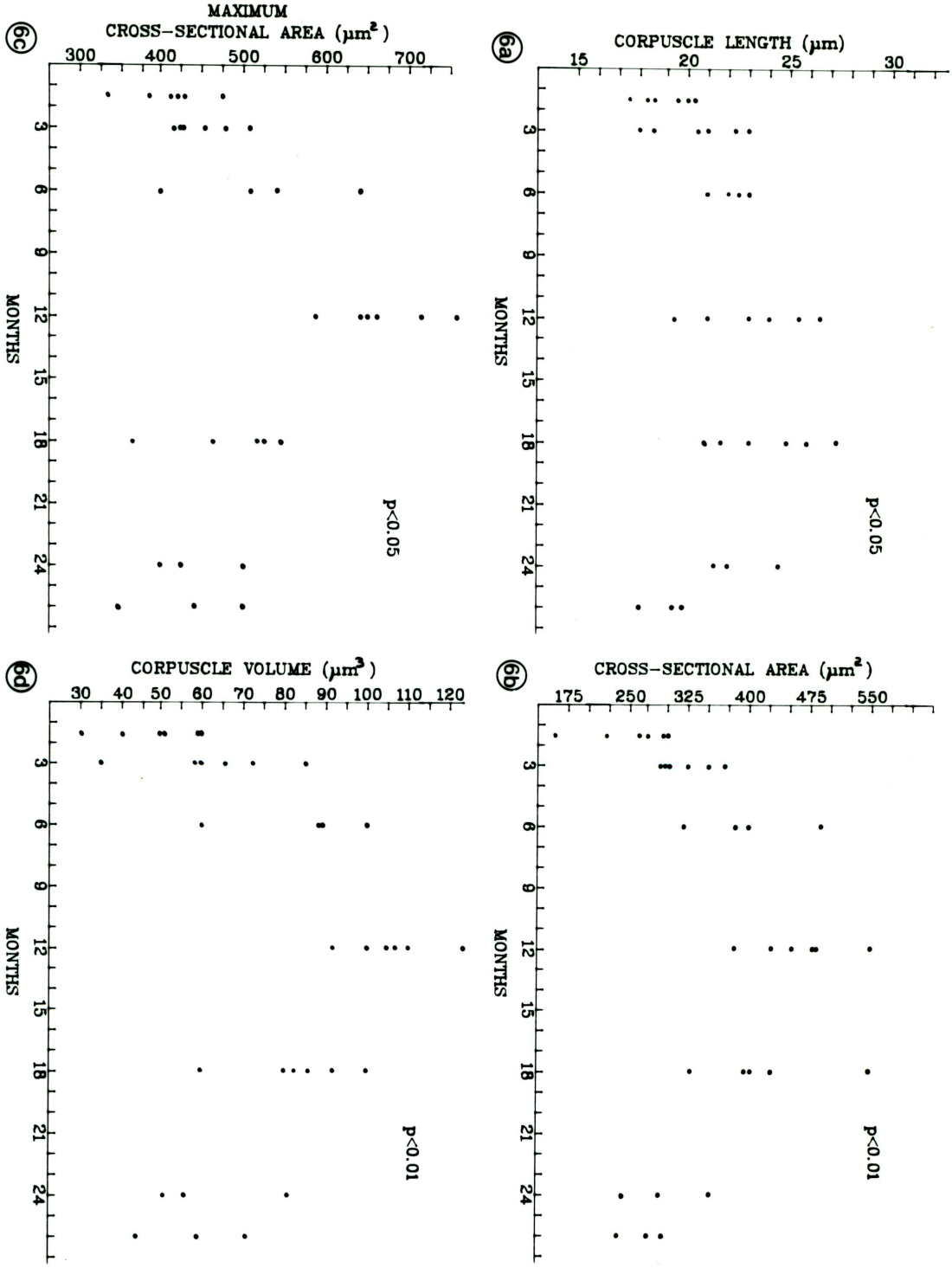
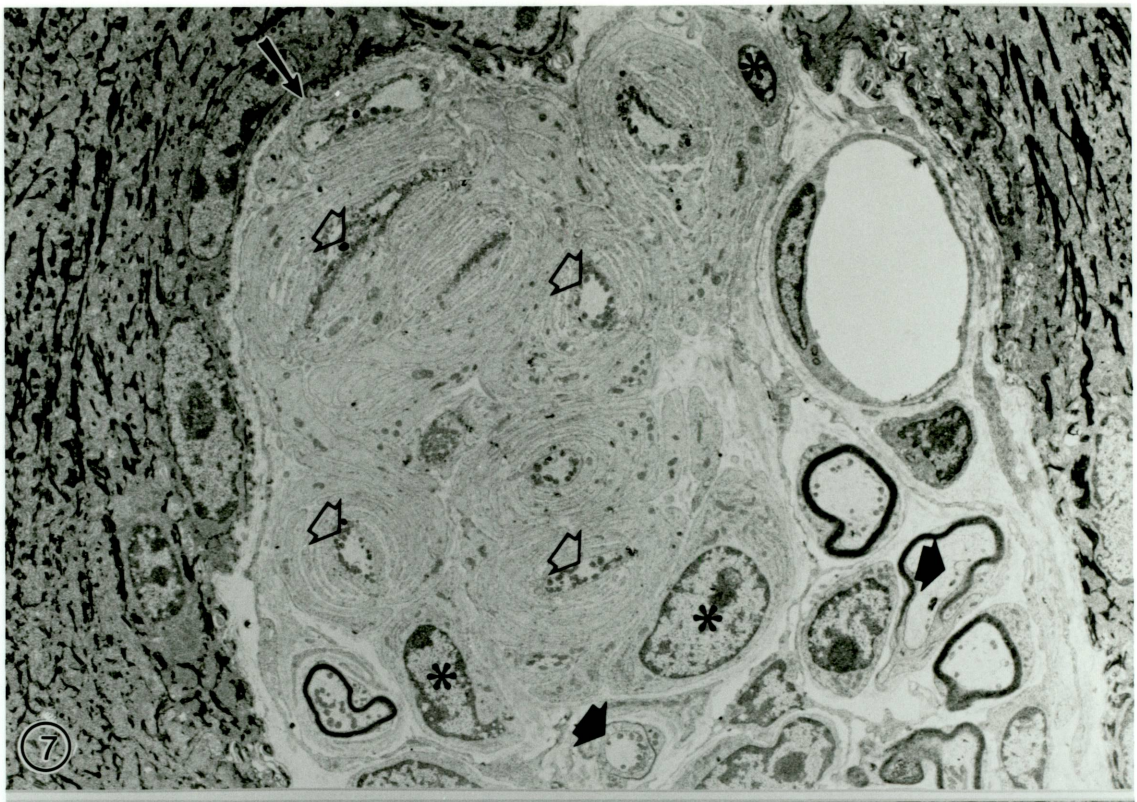

6a
CORPUSCLE LENGTH (μm)
15
20
25
30
MONTHS
3
6
9
12
15
18
21
24
p<0.05
6b
CROSS-SECTIONAL AREA (μm²)
175
250
325
400
475
550
MONTHS
p<0.01
6c
MAXIMUM
CROSS-SECTIONAL AREA (μm²)
300
400
500
600
700
MONTHS
p<0.05
6d
CORPUSCLE VOLUME (μm³)
30
40
50
60
70
80
90
100
110
120
MONTHS
p<0.01

Figure 7: In this corpuscle at age 3 months, several axon profiles are seen as the myelinated extracorpuscular portion (closed arrows) becomes unmyelinated within the corpuscle (open arrows). Three lamellar cell bodies (asterisks) are evident. x5000.

Figure 8: A corpuscle at age 9 months demonstrating well organized structural complexity. Note the increased extracellular material (arrows) as compared to the corpuscle at age 3 months in Figure 6. x6000.

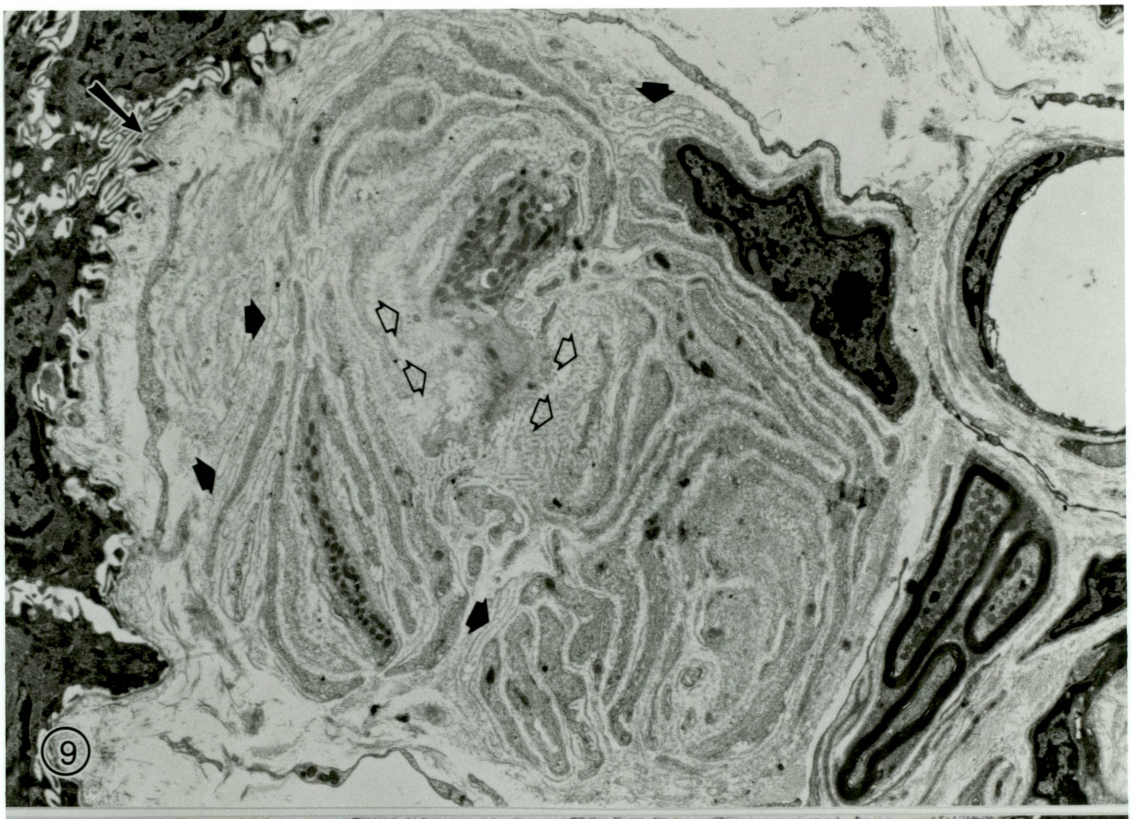


⑦

⑧

Figure 9: In this corpuscle at age 18 months, much more extracellular connective tissue (open arrows) is seen than at younger ages. Fewer lamellae surround the axon profiles and basal lamina duplication (closed arrows) is present. Note the fewer axon profiles as compared to corpuscles at younger ages in Figures 7 and 8. x7500.

Figure 10: An atrophic corpuscle at age 24 months. Note the small size of this corpuscle and the abundant connective tissue (large arrows) filling the space left by attenuated lamellae and axon profiles. No capsule enclosing the corpuscle is observed (small arrows). x8000.



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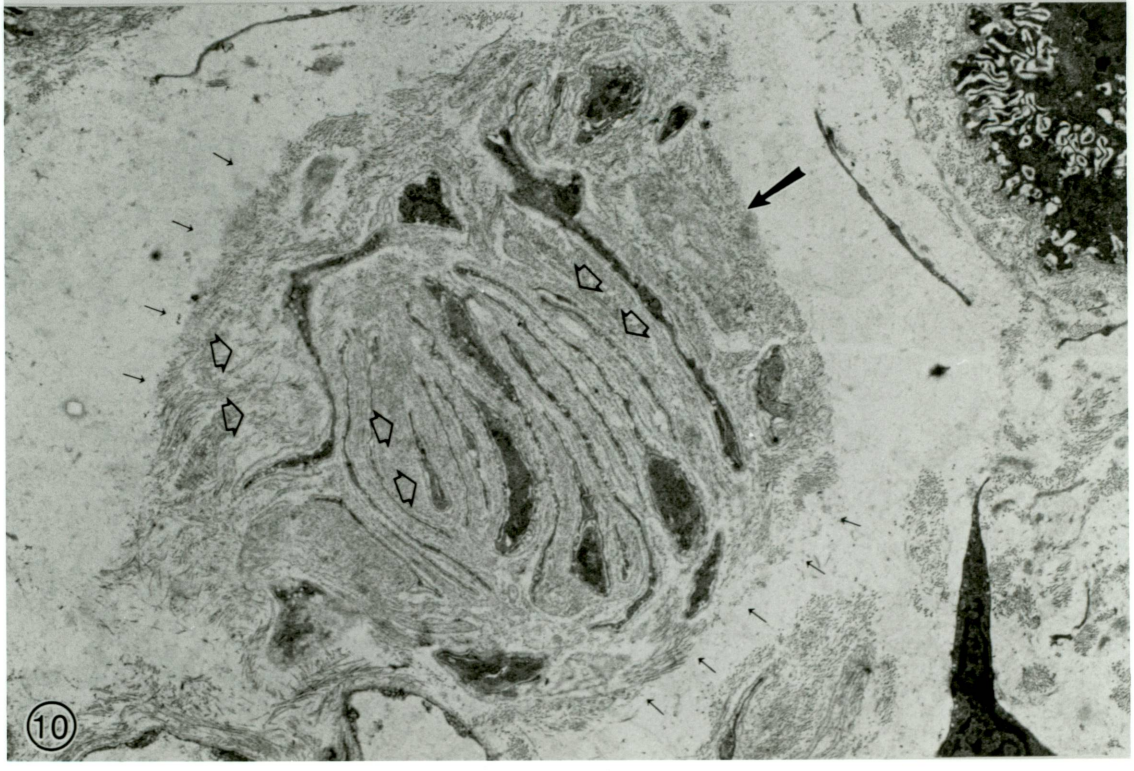
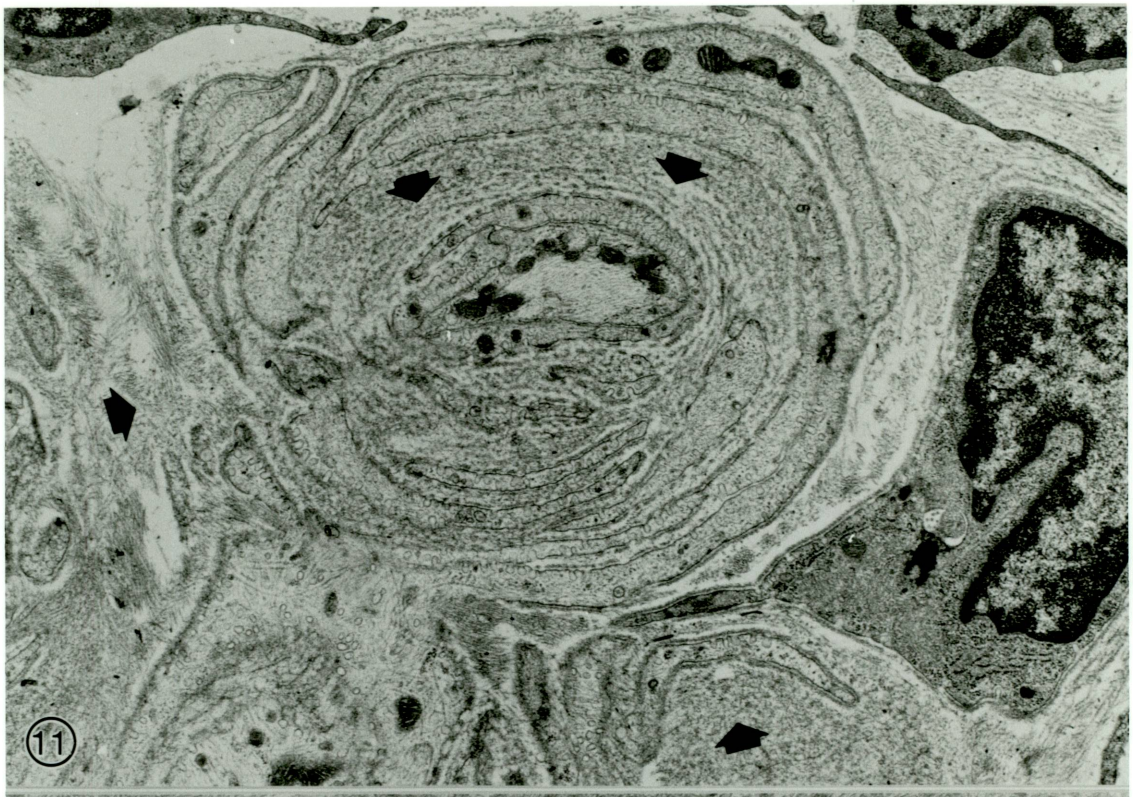


⑩

Figure 11: Electron micrograph of a portion of a corpuscle at 12 months. Note the increased interstitial material (arrows). x17,800.

Figure 12: Electron micrograph of another portion of the corpuscle in Figure 11 showing age-related mitochondrial changes (see Fig. 15). x21,600.



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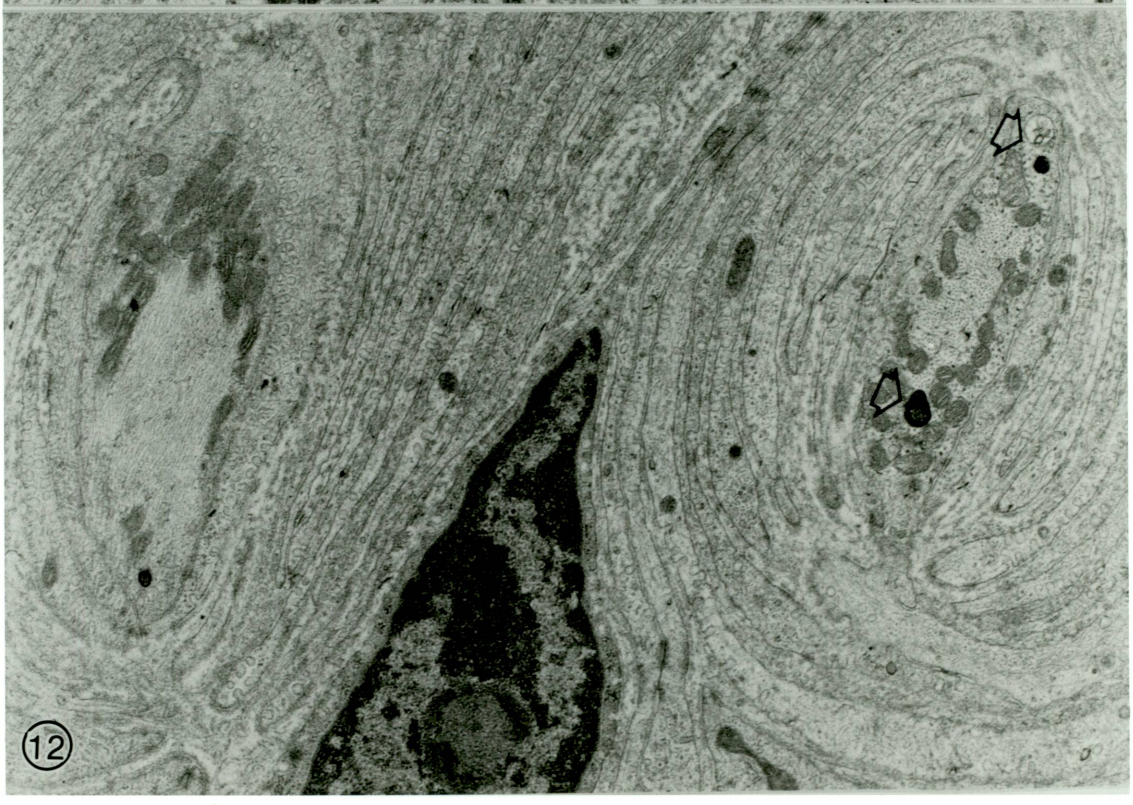
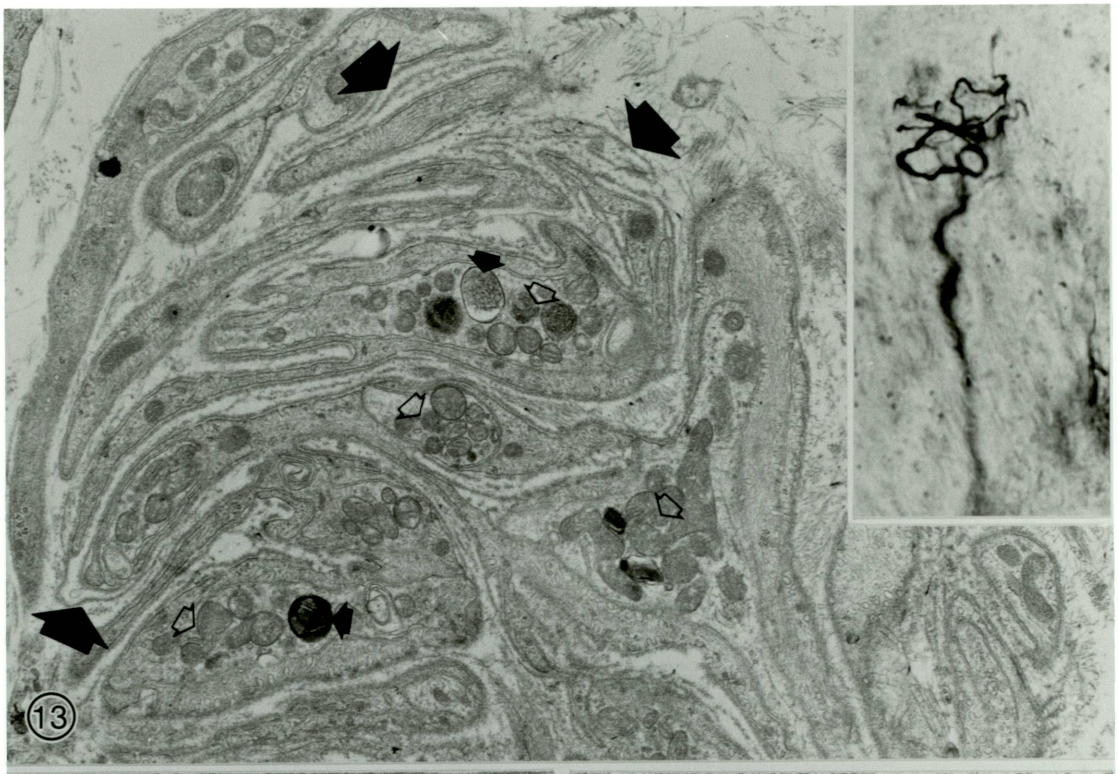


⑫

Figure 13: Electron micrograph of a corpuscle at 12 months having abundant irregular axon profiles containing an unusual arrangement of numerous degenerative mitochondria (open arrows), multivesicular and dense bodies (closed arrows). Only one to three lamellae with few caveoli surround each axon. Basal lamina duplication is present (large arrows). x17,500.Inset: Photomicrograph of silver-impregnated digital pad tissue showing a corpuscle at age 12 months with axons having a character probably similar to that shown in the electron micrograph. (This micrograph was published in J. Comp. Neurol. 1985 and is reprinted with permission of the publisher.) x750.

Figure 14: Electron micrograph of a portion of an atrophic corpuscle at 24 months showing two axon profiles (asterisks) containing an abnormal arrangement of organelles (see text). Few lamellae are present. Lipofuscin is in the lamellar cell cytoplasm (arrows). x23,500.

Figure 15: Electron micrograph of an axon profile and investing lamellae at age 24 months. Degenerative mitochondrial age-changes are exemplified by these organelles becoming dense (open arrows), or dilated and reticulated with lucent matrix (closed arrows). x21,700.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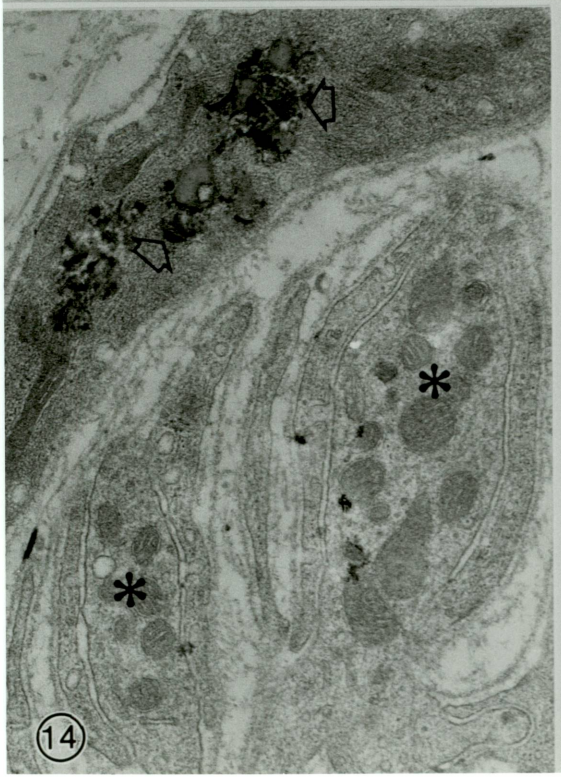
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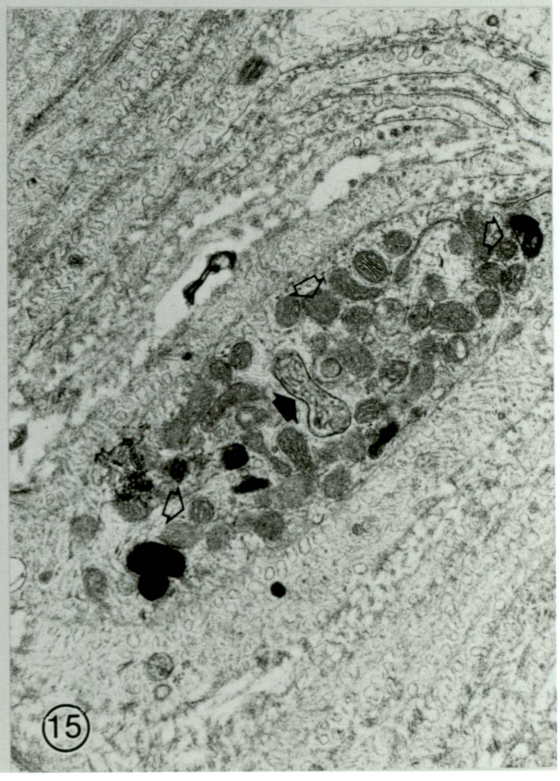
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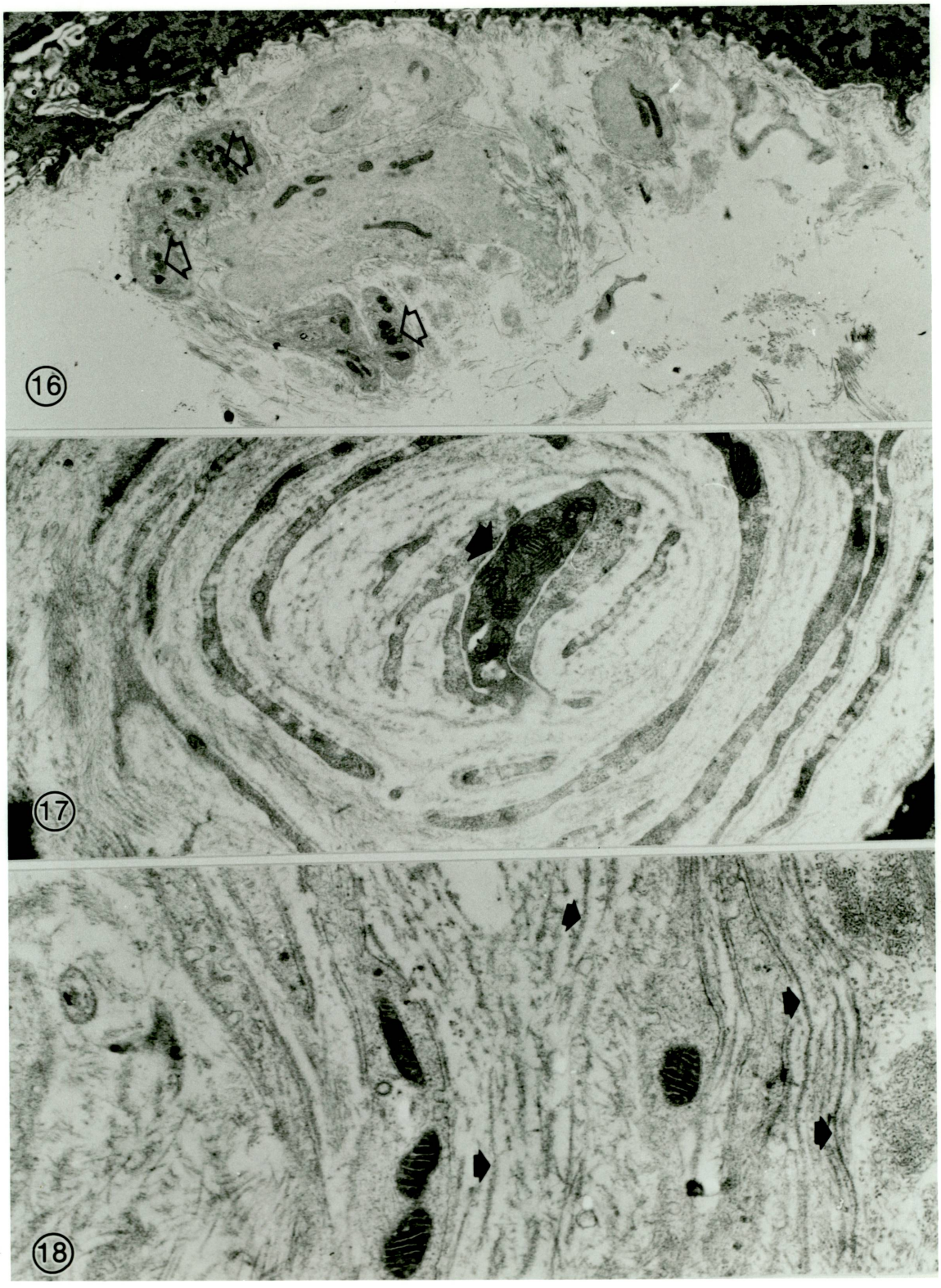
Figure 16: Electron micrograph of the remaining elements of an atrophic corpuscle at age 26 months. Some axon profiles are present (arrows) surrounded by lamellar cell processes and extracellular material. x7100.

Figure 17: Electron micrograph of an axon profile and lamellae at age 26 months. Note the attenuated nature of the axon (arrow) with crowded mitochondria and dense axoplasm. Notice the reduced number of lamellae which are also attenuated and have fewer caveoli than seen at younger ages. Abundant extracellular material is obvious. x18,800.

Figure 18: Electron micrograph of a portion of a corpuscle at age 24 months showing basal lamina duplication (arrows) between remaining lamellae. x23,500.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