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Age-changes of the Neuronal Component of Meissner Corpuscles in the Mouse Digital Pad

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Abstract

AGE-CHANGES OF THE NEURONAL COMPONENT OF MEISSNER CORPUSCLES IN THE MOUSE DIGITAL PAD

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Silver impregnated sections of mouse digital pads were studied using light microscopy to detect age-related changes of the neuronal component of Meissner corpuscles. Direct microscopic observation, photomicrographs and camera lucida tracing were utilized. From qualitative observation, the corpuscular neurites were found to undergo morphological age-related changes of diameter, tortuosity, varicosity, branching and terminal expansion size. Quantitative examination was made of the number of corpuscles, corpuscular neurites, branching neurites, cross-innervations, terminal neurite expansions, neurite intraepidermal continuations and terminal axonal processes. The number of corpuscles and neurite intraepidermal continuations decreased with age while having significant linear correlation; whereas, branching increased with age while having significant linear correlation. Using camera lucida tracings, measurements were made with a Lenz Videoplan computer to determine changes of length and area of the neurites. Significant linear correlation for an age-change of length was not shown, but an

increase with age until 12 months was indicated. However, area showed linear correlation, increasing with advancing age. Physiological correlates of the morphological findings were discussed.

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by

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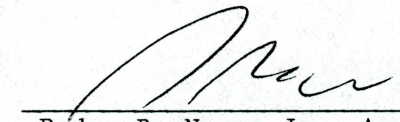

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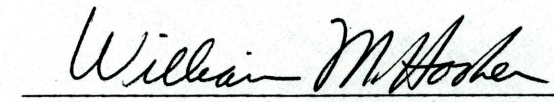

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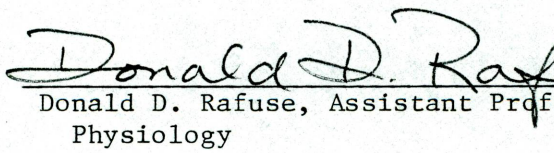

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Key to Symbols of Morphological Characteristics

NIC: neurite intraepidermal continuation; nerve fiber from Meissner corpuscle that penetrates overlying epidermis; shown in figures 1b, 8b and 12.

TAP: terminal axonal process; short neuronal process projecting from a terminal neurite expansion; shown in figure 6.

TNE: terminal neurite expansion; enlarged terminal portion of Meissner corpuscular neurite; shown in figures 4, 5, 6, 9b, 10, 11, 17 and 18.

CI: cross-innervation; neurite(s) that innervate an adjacent Meissner corpuscle by crossing over from one papilla to the adjacent papilla; shown in figures 4 and 10.

EB: epithelial border of dermal papilla; shown in figure 4.

B: branching; shown in figures 4, 10, 11, 12, 16, 17 and 18.

V: varicosity; shown in figures 6, 10, 11, 12 and 17.

Introduction

Age-related loss of cutaneous sensitivity in man has been well documented (Pearson, 1928; Laidlaw and Hamilton, 1937; Steiness, 1957; Whanger and Wang, 1974; and others). Sensitivity to vibration, touch and two-point discrimination decrease with advancing age (Newman and Corbin, 1936; Ronge, 1943; Rosenberg, 1958; Axelrod and Cohen, 1961; Plumb and Meigs, 1961; Verrillo, 1980). The reason for this loss is not clearly understood. Decreased sensitivity could be caused by age-changes in one to several components of the sensory pathway from receptor to cerebral cortex. The focus of this study is on the cutaneous receptor, Meissner corpuscle. Since the Meissner corpuscle contains the terminal portion of the primary sensory neuron dendrite and is trophically influenced by it (Idé, 1982a, 1982b), a review of age-changes found in Meissner corpuscles and other cutaneous receptors would be appropriately preceded by a review of age-changes found in primary sensory neurons.

Decrease in the number of primary sensory neurons may occur with advancing age. Corbin and Gardner (1937) reported a 32% reduction in the number of myelinated fibers in human spinal roots with increasing age, and Gardner (1940) found a similar decrease in the number of human spinal ganglion cells. Age-related 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 but found 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 a possible reduction in the number of human unmyelinated fibers with age (Behse, et al., 1975).

Age-changes of the diameter of peripheral nerve fibers in general could suggest diameter changes of the sensory nerve fibers contained in the peripheral nerve. Sharma, et al. (1980) found in young rat tibial and plantar nerves an initial rapid increase in fiber diameter followed by a more gradual increase, which ceased after approximately 9 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

Motor impulse conduction velocity changes evidenced by motor responses may occur with age. The maximum velocity of human medial plantar nerve fibers for 50-69 year old humans dropped by 10m/s when compared to 10-29 year olds (Sommer, 1941). Wagman and Lesse (1952) found a drop of velocity in human ulnar nerve fibers of about 7m/sec between the third and seventh decade. Norris, et al. (1953) reported a drop of velocity in human ulnar nerve fibers of about 10m/sec from the ages of 30-39 to ages 80-89. Birren and Wall (1956), however, report no significant change of conduction velocity in rat sciatic nerve fibers. If motor impulse conduction velocity changes with age, probably sensory impulse conduction 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 in 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 signs of structural decay in peripheral nerve fibers have been defined by many others (Spencer and Ochoa, 1981).

While the primary sensory neuron has been shown to undergo age-related changes, studies have also shown morphological changes of cutaneous receptors to occur with advancing age. Cauna (1965) found that in humans some Pacinian corpuscles increased in size and others were remodelled or reformed on the axons of obsolete corpuscles; Merkel's and Meissner corpuscles were progressively reduced in number. He discovered that Meissner corpusles gradually lost their epithelial attachment, moved deeper into the dermis, increased in length, and became coiled and lobulated to a varying degree. The neurite terminations in Meissner corpuscles changed from the original neurofibrillar networks of young persons into coarse, winding terminal expansions in adult male fingers and toes of both sexes. In the elderly, the neurite terminations were composed of fine winding filaments of irregular arrangement. Schimrigk and Ruttinger (1980) performed a study of Meissner corpuscles in the human big toe with respect to age, and their conclusions were basically the same as those made by Cauna (1965). In addition, they determined that Meissner

corpuscles decrease in number exponentially. During the first decades of life, they found the corpuscles to exhibit lateral and longitudinal growth. At advanced age they found atrophy to occur, evidenced by a decrease in number and size of corpuscles. With increasing age the corpuscles became less uniformly distributed across the histological specimen (skin). They also discovered that certain diseases, particularly disorders resulting in polyneuropathies, could initiate changes of the number and structure of the corpuscles. Other studies describing age-related changes of Meissner corpuscles have produced similar results (Perez, 1931; Ronge, 1943; Dickens, et al., 1963; Bolton, et al., 1966; Hunter, et al., 1966; Hunter, et al., 1969; Kastowiechi, 1971; Bruce, 1980).

In another study a comparison was made between the corpuscles of elderly people and the corpuscles of people with neurological diseases (Dyck, et al. 1966). Since age-related and age-unrelated pathology do not necessarily coincide, important is the attempt to differentiate between the effects these two separate phenomena may have on morphology. Understanding age-related changes of Meissner corpuscles will set a comparative base line for studying neuropathological changes in these corpuscles not caused by aging. Also age-induced morphological changes of these receptors, if found, may explain decrease of cutaneous sensation with advancing age. While Meissner corpuscles have been shown to undergo age-related changes, few detailed morphological studies have been done to determine exactly what the qualitative and quantitative age-related changes are. Electron microscopic studies as well as more light microscopic studies can be done to describe specific aspects and parts of

the corpuscle that change.

The development (Idé, 1977, 1982a) and fine structure (Idé, 1976), as well as the degeneration and regeneration of the mouse digital corpuscle have been investigated (Idé, 1982b, 1982c). But no studies have been performed which describe changes in mouse digital corpuscles from maturity to old age. Macintosh and Sinclair (1978) performed a related study of age-related changes in the innervation of the rat snout and found the corpuscle nerve endings change in a manner similar to aging human Meissner corpuscles. Since Idé (1976) has shown that mouse digital corpuscles are essentially the same structures as human Meissner corpuscles, excluding the small size of mouse digital corpuscles, an age-related study of mouse digital corpuscles would add information about aging Meissner corpuscles. The mouse would provide a practical model for aging in mammals because of its small size, relatively brief life span and easy attainability for laboratory studies. Swiss Webster albino female mice (from Brookhaven National Laboratory) have a mean survival time of 16.35 months, a maximum survival time of approximately 28 months and a normal pathology indicative of senescence (Johnson and Cronkite, 1967). Therefore, albino mice are a good choice as laboratory animals for aging studies.

No study has been done to analyze age-related changes of the neuronal component of Meissner corpuscles in detail although a few age-changes of the neurite have been noted as mentioned previously. Therefore, this is a study of age-related changes of the neuronal component in digital Meissner corpuscles of the albino mouse.

Procedure

Swiss Webster albino female mice from Simonsen Laboratory were maintained at 24° C with four to seven mice per shoebox cage. The daily light/dark ratio was 12 hrs/12 hrs. Fresh bedding (wood shavings) was provided at biweekly intervals and a standardized nondeficient diet (Rodent Laboratory Chow 5001) and water were given ad libitum. Cages were washed once a month and water bottles were washed once a week. Mice were sacrificed at ages 1.5, 3, 6, 9, 12, 15, 18, 21 and 24 months. Six mice at each age, making a total of 53 mice (only five mice were 24 months), were processed for light microscopy. Animals with gross pathological abnormalities were not used.

After obtaining body weights and noting any obvious pathology, mice anesthetized with Nembutal (sodium pentobarbital, dosage: 50 mg/kg of body weight by intraperitoneal injection) were perfused through the heart with 10% unbuffered formalin. The forepaws were then cut off and immersed in the formalin for several days. Digital pads (total of 4 per paw) containing the receptors were excised using a razor blade. Tissues were dehydrated and rehydrated in a graded series of denatured alcohol. Thirty micron serial sections of the digital pads were cut perpendicular to the skin surface and parallel to the digit. Tissues were silver impregnated according to the Winkelmann and Schmit (1957) technique. Impregnation solutions were added and then decanted off from each beaker containing sections from one digital pad. Sections representing a single digital pad were mounted on one glass slide without preserving serial arrangement. For sections chosen and analyzed for age-related changes, more than two

sections were rarely chosen from the same digital pad. When there was difficulty finding sections with good impregnation representing a single mouse, more than two sections may have been chosen from the same digital pad.

Twenty photomicrographs and twenty camera lucida tracings were made of representative corpuscular neurites from mice of each age group. These were evaluated qualitatively for morphological age-related changes. For analyzing the tortuosity of the neurites, two subdivisions were created: primary tortuosity and secondary tortuosity. Primary tortuosity described the spiraling of the neurite that occurred without causing major change to its orientation. Secondary tortuosity described any looping, twisting or bending of the neurite that caused major change of its orientation.

Meissner corpuscles were identified by the unique character of the nerve ending. The neurite as it courses through the corpuscle gains a horizontal orientation to the skin surface by twisting and bending. Since sections were approximately the same thickness as the corpuscles, a three-dimensional effect was created when focusing up and down through each section, allowing the tortuosity of the neurites to be easily visualized. By microscopic examination, counts were made of the number of Meissner corpuscles per section. For these counts each age group was represented by 50 sections that had two characteristics - showing good impregnation and representing the approximate center of a digital pad.

For quantitatively determining if any morphological age-related changes occurred, sections from the center of the digital pads with good impregnation quality were selected for counts of the number of neurites per corpuscle, branches per corpuscle, cross-innervations (CI's) per

section, terminal neurite expansions (TNE's) per corpuscle, neurite intraepidermal continuations per section and terminal axonal processes per section. Since nerve fibers may have more than one branch and more than one TNE, counts were made of the number of nerve fibers having one branch, more than one branch, one TNE and more than one TNE. The number of nerve fibers involved in CI's was also noted. The percentage of corpuscles per section having each of these characteristics was calculated for comparative purposes. Changes in length and area were approximated by using a Lenz Videoplan computer to measure camera lucida tracings. One neurite from each corpuscle was selected at random and measured. The length was measured by tracing the neurite's tortuous course to its termination with the Videoplan stylus. The point to start the length measurement on the neurite tracing (x2400) was determined by making a linear 100mm measurement (actual size of a 100mm measurement at 2400X magnification is 40μ) from the termination of the neurite. For calculation of area, the perimeter of the neurite within the same linear interval was traced with the Videoplan stylus so that the Videoplan computer could calculate the area. Reference to examples of the characteristics analyzed for age-related changes are given on page vi.

A "mean" for each corpuscular characteristic quantitatively analyzed for age-related changes was calculated. A "mean" delineating each corpuscular characteristic for each individual mouse could not be calculated for mice 1.5 to 12 months of age because specimens were not identified according to individual, just age; therefore, characteristics from these mice were represented by "group means" calculated from data taken from each age group of six mice without knowing which mouse

contributed which data. However, a "mean" delineating each characteristic for each individual mice 15 to 24 months of age could be calculated; a "mean of means" was then calculated for these mice. Regression and linear correlation statistics detected if any significant age-dependent changes occurred.

Results

I. Qualitative observations:

Regardless of age, all corpuscular neurites examined maintained two essential characteristics: a tortuous course and a location in dermal papillae. Other neurite characteristics did not occur consistently and had considerable morphological variation within each individual mouse and each age group of mice. Although this variability made detection of age-related changes to be difficult, some changes appeared to occur. For a description of these changes, the lifespan of the mouse was divided into three phases: young adult (1.5 to 6 months old), middle adult (9 to 15 months old) and old adult (18 to 24 months old).

1.5 to 6 months old (fig's. 1-6)

At six weeks (fig's. 1 and 4), most corpuscular neurites appeared relatively attenuated with little varicosity and primary tortuosity. However, a large degree of secondary tortuosity was evident. Many of the corpuscules were innervated by one to several very thin neurites in addition to the one or two neurites with normal diameter that usually innervated the corpuscles. Neurite intraepidermal continuations (NIC's) were frequently seen penetrating the overlying epithelium (fig. 1b). Terminal neurite expansions were infrequent and relatively small (fig's. 1a and 4). Neurite branching was detected in many of the corpuscles (fig. 4, drawing 1) but the ramification had little complexity. In some instances, cross-innervation (CI) was noted (fig. 4, drawing 1), but terminal axonal processes (TAP's) were rarely seen.

A few age-related morphological changes occurred between corpuscular neurites of mice 1.5, 3 and 6 months old. Although neurites appeared attenuated at 1.5 months (fig. 1 and 4), at 3 months (fig's. 2 and 5) and especially 6 months (fig. 3 and 6), the neurites appeared larger in diameter. Primary tortuosity increased slightly at 6 months (fig. 6), secondary tortuosity likewise at 3 months (fig. 5). More large TNE's were encountered at 6 months (fig. 6).

9 to 15 months old (fig's. 7-12)

Neurite morpholgical differences were detected between corpuscles in young mice and corpuscles in middle aged mice. The average diameter of neurites increased (compare fig's. 4-6 with 10-12) and TNE's were larger and more abundant. There was an obvious increase in primary tortuosity (fig. 7a) and a less obvious increase in secondary tortuosity. This increased tortuosity possibly gained more horizontal orientation for the neurites, exemplified by comparing figure 4-6 with figures 10-12. In addition, the area occupied by the neurites course appeared larger than at younger age. Varicosities (fig. 10, drawing 2) and short branches (fig. 10, drawing 4), found at irregular intervals along the neurite, were more frequent. Especially at 15 months, more corpuscles had fine filamentous branches extending from terminal portions of the neurites (fig. 12, drawing 1). Neurite ramification seemed more numerous; however, NIC's were rarely encountered (fig. 8b). The complex appearance of the corpuscular neuronal component seemed increased by these morphological changes.

Between ages 9, 12 and 15 months, a few changes were observed. Primary tortuosity appeared highest at 9 months (fig's. 7a and 7b), having

declined at 12 and 15 months (fig's. 8a-9b). Secondary tortuosity increased between ages 9 and 12 months (compare fig's. 7b and 8b), but decreased at 15 months (fig. 9b). Due to this change in tortuosity, the neurites courses appeared most horizontally oriented at 12 months (compare fig's. 10,11 and 12). The average size of TNE's appeared to increase from ages 9 to 12 months with no change at 15 months. A climax in size of the area occupied by the branches and tortuous corpuscular neurites, as well as the appearance of complexity resulting, occurred at 12 months (compare fig's. 10,11 and 12).

18 to 24 months old (fig's 13-18)

Neurites from corpuscles in old mice were similar to those in mice of middle age, thus indicative that few morphological age-related changes occurred between middle age and old age. However, some subtle differences did exist. At old age, neurites appeared more attenuated similar to neurites at 1.5 months (compare fig. 1a with 15a). Primary tortuosity seemed decreased, although secondary tortuosity seemed unchanged (compare fig. 7a with 18). Apparently, the tortuosity changed into a more random arrangement rather than the more regular horizontal arrangement seen in younger corpuscles well exemplified by the left corpuscle in drawing 3 of figure 11. In addition, the size of TNE's decreased as well as the number of varicosities (fig's. 17 and 18).

Changes of the corpuscular neurites between the three different age groups (18,21 and 24 months) did occur. At 24 months and exemplified well by figure 18, neurites were clearly most attenuated and TNE's were fewest in number and small; also receptor size decreased as indicated by the

extent of areas occupied by entangling neurites (fig. 18). Primary tortuosity appeared least at 24 months (compare fig's. 16,17 and 18) and clearly the number of corpuscles per digital pad appeared lowest (fig. 15). Interestingly, at 24 months empty papillae were most frequently encountered (fig. 15b).

Summary (1.5 to 24 months old)

Obvious age-changes observed in the corpuscular neurites are illustrated in figure 19. Neurite thickness and tortuosity increased until age 12 months. From age 12 to 24 months, neurite thickness decreased. The size of the corpuscle, evidenced by the length and width of the area containing the portion of the neurite with corpuscular characteristics, increased until age 12 months and decreased after 12 months.

II. Quantitative observations:

Light microscopic examination. Tables 1 through 5 show for each age group of mice the mean values for density of Meissner corpuscles per section, number of neurites innervating each Meissner corpuscle, and each of the neurite morphological characteristics analyzed for age-related changes.

Measurement of camera lucida tracings. Mean values for length and area of the Meissner corpuscular neurites representing each age group of mice are given in table 6.

Statistical analysis. Linear regression and correlation test results are shown in table 7 and figures 20 through 36. The statistical parameters for each characteristic of the Meissner corpuscle are listed in table 7

according to the order of best to least linear-regression correlation.

Corpuscle concentration: As shown by the graph in figure 20, the number of Meissner corpuscles per section (MC/S) decreased significantly with age (slope of linear-regression line =-0.31). However, an increase of corpuscle number occurred from ages 1.5 months to 3 months. The high correlation (table 7) indicates that the decrease of corpuscle number that occurred after 3 months was fairly linear. Corpuscle numbers decreased approximately 70% between ages 3 and 24 months.

Innervation: The number of neurites innervating each Meissner corpuscle (N/MC) changed with age in a similar pattern as corpuscle concentration (fig. 21), having increased from ages 1.5 months to 3 months and steadily decreased after 3 months. The correlation to linear regression, however, was not significant, even at the 0.05 confidence limit (table 7). The degree of innervation change with age is indicated by the slope of the linear-regression line (table 7).

Branching: The number of branches per corpuscle (B/MC) in each section increased with age (fig. 22). The correlation to linear regression was good (table 7). An age-related change in the number of corpuscular neurites with more than one branch (N$\bar{c}$>1B) was not too evident (fig. 22), especially as shown by the bad correlation (table 7); although, the slope of the regression line does increase (fig. 22 and table 7). A good correlation was indicated for a positive linear regression between the percentage of Meissner corpuscles per section having branches (%MC$\bar{c}$B) and age (fig. 24 and table 7).

Cross innervation: No age-dependence was detected with cross-innervation measurements (fig. 25, 26 and table 7); although, there

was a negative slope of the linear-regression lines both characteristics analyzed - number of cross innervations per section (C1/S) and percentage of Meissner corpuscles per section with cross-innervations (%MC$\bar{c}$CI).

Terminal neurite enlargements: No correlation was detected for a linear regression between terminal neurite enlargements (TNE's) and age (fig. 27, 28, 29, 30) and table 7). However, the slope of the linear-regression line of each characteristic analyzed (MC$\bar{c}$1TNE, MC$\bar{c}$>1TNE, TNE/MC and %MC$\bar{c}$TNE) was consistently negative (table 7).

Neurite intraepidermal continuation: The number of Meissner corpuscles per section with neurite intraepidermal continuations (MC$\bar{c}$NIC) decreased with age (fig. 31) and significant correlation with negative linear regression was indicated (table 7). An age-related decrease in the percentage of Meissner corpuscles per section with neurite intraepithelial continuations (%MC$\bar{c}$NIC) was also shown (fig. 32) with correlation to linear regression at the 0.05 confidence limit (table 7).

Terminal axonal processes: No linear-regression correlation was detected (fig. 33, 34 and table 7).

Area: The area of a camera lucida tracing (x2400) of the neurite over a 100mm linear interval extending from the termination of the neurite increased with age (fig. 35). A correlation to linear regression did exist at the 0.05 confidence limit (table 7).

Length: No linear-regression correlation was detected for length of the tortuous corpuscular neurite over a 100mm linear interval. The slope of the linear-regression line was positive (fig. 36 and table 7).

Discussion

Changes with advancing age were detected in the following characteristics: number of Meissner corpuscles per tissue section, number of neurites innervating each corpuscle, neurite ramification, terminal neurite enlargement size, number of neurite intraepidermal continuations, neurite area, neurite width, neurite tortuosity and neurite varicosity. No strong evidence was found for changes with age in the number of cross-innervations, terminal neurite enlargements and terminal axonal processes.

Clearly the most pronounced age-related change was an approximate 70% decrease in the number of Meissner corpuscles between ages 3 months and 24 months. This decrease of corpuscle concentration in mice agrees with prior studies on humans (Perez, 1931; Ronge, 1943; Dickens, et al., 1963; Bolton, et al., 1966; Cauna 1965; Hunter, et al., 1969; Kastowichi, 1971; Bruce, 1980; Schimrigk and Ruttinger, 1980). Atrophic changes of Meissner corpuscles immediately preceding their loss were difficult to detect in the present study because tissues were not stained so that all corpuscular elements would be seen. Atrophy of these unstained corpuscular elements was therefore undetectable. Atrophic human Meissner corpuscles have been described as abnormally narrow and faintly stained, having few irregularly arranged nerve terminals which are surrounded by cells with pyknotic nuclei (Cauna, 1965 and Schimrigk and Ruttinger, 1980). A major cause of an age-related corpuscle atrophy and resulting receptor loss may be denervation. In mice when the nerve supply is interrupted proximal to the

Meissner corpuscle, degenerative changes occur in the receptor (Ide, 1982a). These mouse corpuscular changes were similar to the age-associated atrophic changes found in human Meissner corpuscles, although the mouse was described at the electron microscopic level; whereas, the human was studied at the light microscopic level. If denervation is the major cause of decreasing numbers of Meissner corpuscles with advancing age, what is causing the denervation? The reported age-related degeneration and loss of primary sensory neurons is a likely reason.

Between ages 1.5 and 3 months, corpuscles numbers increased (8.7 MC/S to 10.2 MC/S); however, this increase was small (1.5 MC/S) and possibly not significant. But if this increase in corpuscle concentration was significant, then new corpuscles must have formed. Interestingly, at 1.5 months there were many neurites with no corpuscular characteristics found innervating the digital pads. Perhaps some of these neurites represented undeveloped Meissner corpuscles that would become corpuscular by age 3 months. New Pacinian corpuscles form on the proximal part of axons already innervating Pacinian corpuscles (Cauna, 1965). If new Pacinian corpuscles can form during the lifetime of an individual, maybe new Meissner corpuscles can form.

Decrease with age of corpuscle concentration does not appear to be the only reason for a decrease with age of discriminative touch sensation; in fact, touch thresholds and corpuscle concentrations do not correlate even at the same age when comparing different areas of skin (Bruce, 1980 and Verrillo, 1980). For example, the dorsal skin of human fingers has fewer Meissner corpuscles per unit area than the lateral skin, yet touch

detection thresholds at these sites do not differ significantly; no significant difference exists between the population sizes of corpuscles in palmar and lateral sides of the fingers, yet the palmar skin is significantly more sensitive to touch (Bruce, 1980). If corpuscle concentration and touch thresholds do not coincide, other factors beside corpuscle concentration must be involved to account for changes of sensitivity between different areas, and likewise, different ages of skin.

Although not significant as a linear regression, the number of neurites innervating each corpuscle were shown to decrease with advancing age. Perhaps this indicates that nerve fiber population is decreasing with age as indicated by nerve fiber counts in peripheral nerves. Such a decrease of nerve fibers could also be a cause of declining sensitivity with age. Neurite numbers, however, increased between age 1.5 and 3 months. Interestingly, this increase correlates with the increase of corpuscle concentration noted between these same age groups, more evidence for suggesting that new Meissner corpuscles develop between 1.5 and 3 months of age.

Cauna (1965) has suggested that Meissner corpuscles are dynamic structures, changing morphologically throughout the lifespan of an individual. The present study supports this idea. Evidently two processes seem to be simultaneously occuring in the population of these receptors - development and degeneration. Development between 1.5 and 3 months has been indicated by an increase of corpuscle concentration and neurite numbers. Degeneration after 3 months has been indicated by decrease of corpuscle concentration and neurite numbers. These two changes suggest that development predominates before 3 months, and

degeneration predominates after 3 months. However, the other changes that occurred with age in these mouse corpuscular neurites do not follow the same pattern. Some changes indicated that development continued beyond 3 months: increase of number and size of terminal neurite expansions until age 12 months, increase of tortuosity also until 12 months, and increase of branching up until 21 months. Yet, these developmental changes occurred along with degenerative changes: decrease of corpuscle concentrations, decrease of neurite numbers, decrease of the neurite's regular horizontal arrangement (after 12 months), decrease of the size of terminal enlargements (after 18 months).

The most distinct characteristic of Meissner corpuscular neurites is their tortuosity. As the neurite bends and twists along its course within the corpuscle, it gains horizontal orientation to the skin surface. This orientation of the neurite is probably necessary for the mechano-electric transducing function of the receptor (Idé, 1976 and Nishi et al., 1969). Age-related changes of the neurites' tortuosity may therefore have functional significance. Primary tortuosity appeared to increase till age 9 months, then a gradual decrease was noted from age 9 months to 24 months. Secondary tortuosity showed greatest increase from 1.5 to 3 months, appeared in highest magnitude at 12 months, and showed tendency toward randomness at advanced age. An increase or decrease of tortuosity would likewise increase or decrease the length of neurite in the corpuscle. Therefore, an age-change of length should correlate with an age-change of tortuosity. Length did not have a linear correlation with age but was found to increase nearly linearly till 12 months of age. Greatest increase was between 1.5 and 3 months which corresponds with the

high increase in secondary tortuosity between these ages. The maximum of length at 12 months corresponds with the maximum of secondary tortuosity at 12 months. There seems to be no correlation between length and tortuosity after 12 months. But it probably can be safely justified that tortuosity is increasing between ages 1.5 and 12 months. Tight spiraling of the neurite increases until 9 months (primary tortuosity). Bending, coiling, and looping of the neurite (secondary tortuosity) increases until 12 months. This increase of tortuosity until 12 months indicates that the corpuscular neurites are undergoing a developmental change, i.e. the extent of neurite length oriented parallel to the skin surface is increasing. Possibly the corpuscle is compensating for a loss of function with advancing age. After 12 months, maybe this compensatory mechanism is lost.

Neurite branching and terminal neurite enlargements, like tortuosity, probably serve to increase the proportion of neuronal component oriented parallel to the skin surface. Age-related changes of these two characteristics could therefore be important. Significant linear correlation with age was shown for an increase in the number of branches per corpuscle and the percentage of corpuscles having branches; however, no significant linear correlation was shown between age and the number of neurites having more than one branch. Evidently increasing numbers of neurites are ramifying but individual neurites are not increasing their degree of ramification, at least not linearly with age. Terminal neurite expansions (TNE's) did not show any significant linear correlation with age. However, each of the four different counts (neurites with one TNE, neurites with more than one TNE, TNE's per corpuscle, and percentage of

corpuscles having TNE's) consistently had a negative slope when plotted against age, strongly suggesting a decrease in the proportion of corpuscular neurites having TNE's. Loss of TNE's suggests degeneration of the neuronal component's size and possibly its functional capability. The TNE, also called the ultraterminal, has been postulated to be the site of mechano-electrical transduction just as the ultraterminal in Pacinian corpuscles (Idé, 1976). In both receptors the ultraterminals are oriented so that the long axis is parallel to the skin surface and the minor axis is perpendicular to the skin surface. In Pacinian corpuscles pressure directed along the minor axis elicits mechano-electrical transduction (Nishi et al., 1969). If these mechano-transducing elements of the Meissner corpuscle are lost with age, then the receptive capability may be decreased. Another consistency of the four different TNE counts was an increase of all four counts between 1.5 and 3 months, strong evidence for a rise in TNE number during this age interval. This is another indicator that corpuscle development was dominating the morphological changes occuring between 1.5 and 3 months.

Growth or degeneration of the neuronal component in Meissner corpuscles might be indicated by an increase or decrease, respectively, of the volume occupied by the neurites. In this study, area, as could be measured from a camera lucida tracing of the neurite, was used as an approximation of volume. Correlation was shown for a linear increase of area with advancing age at the 0.05 confidence limit. Area increased most obviously between ages 1.5 and 6 months, strongly suggesting growth of the neurites at these younger ages. The neurites were observed to increase in thickness greatly by 6 months and TNE's were increased in size as well,

thus supportive of growth having occurred. This increase of area with age, however, gives little indication that degeneration coexisted with this growth and dominating any changes of the neurites at older age. Maybe an area decrease was not a good indicator of degeneration. However, it seems inconsistent that the neurites were observed to become attenuated at extreme old age (21 and 24 months) and had smaller TNE's, yet the area showed no decrease at these ages.

No functional significance has been conjectured for neurite intraepidermal continuations (NIC's). Cauna (1965) and Idé (1976, 1977) have observed the presence of NIC's and have noticed their occurence to be highest at a young age. Perhaps NIC's are a specialization occurring in some receptors that enhances the sensitivity of these receptors. A decline of occurence of NIC's may cause a decline of sensitivity. In the present study, NIC's decreased in number with advancing age with a significant linear correlation.

The observation of cross-innervation (CI) gives evidence to the idea that more than one corpuscle is innervated by a single primary sensory neuron. CI has been observed in the innervation of taste receptors (Miller, 1974). If an age-related change occurs in the frequency of CI, perhaps this indicates a decrease in size of the receptive field of a primary sensory neuron. However, no significant age-related change was found for numbers of CI's in the present study.

Axonal processes have been observed on the ultraterminal in Pacinian corpuscles and were postulated to function for detecting pressure transients transmitted through the outer core of the corpuscle to the specialized basal regions of the axonal processes where mechano-electric

transduction may occur (Spencer and Schaumberg, 1973). Perhaps terminal axonal processes (TAP's) have a similar function in Meissner corpuscles. TAP's in this study, however, were observed very infrequently, thus suggesting that they are not essential to the function of Meissner corpuscles. Also no significant age-related change was found in their occurrence. TAP's should not be confused with the axonal microprocesses of mouse digital corpuscles described by Idé (1976) which are smaller and not able to be seen with the light microscope. Perhaps axonal microprocesses are the analog of axonal processes in Pacinian corpuscles. TAP's may be infrequently occurring aberrations of the TNE with no functional significance.

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TABLES

TABLE 1

AVERAGE VALUES FOR EACH AGE OF MICE FOR THE NUMBER OF MEISSNER CORPUSCLES(MC) PER TISSUE SECTION(S) AND NEURITES(N) PER MC.

Age(months)	MC/S	N/MC
1.5	8.7	1.6
3	10.2	2.5
6	9.4	2.0
9	9.1	2.0
12	7.9	1.7
15	6.8	1.7
18	5.1	1.3
21	4.1	1.8
24	2.6	1.3

TABLE 2

AVERAGE VALUES FOR EACH AGE OF MICE FOR THE NUMBER OF BRANCHES(B) PER MEISSNER CORPUSCLE(MC), NEURITES (N) IN EACH TISSUE SECTION(S) WITH MORE THAN ONE B. AND PERCENTAGE OF MC's IN EACH S WITH B's.

Age(months)	B/MC	N≥B	%MC≥B
1.5	0.50	0.6	29
3	0.56	0.9	35
6	0.64	1.1	39
9	0.40	1.9	23
12	0.92	1.6	44
15	0.66	0.9	41
18	1.2	1.5	60
21	1.4	1.6	61
24	1.2	1.0	53

TABLE 3

AVERAGE VALUES FOR EACH AGE OF THE NUMBER OF CROSS-INNERVATIONS(CI) PER TISSUE SECTION(S), AND PERCENTAGE OF MEISSNER CORPUSCLES(MC) IN EACH S WITH CI's.

Age(months)	CI/S	%MC≥CI
1.5	0.6	12
3	1.3	14
6	0.4	8
9	0.5	9.5
12	0.5	7
15	0.6	11
18	0.0	0
21	0.3	17
24	1.1	5

TABLE 4

AVERAGE VALUES FOR EACH AGE OF MICE FOR THE NUMBER OF NEURITES (N) IN EACH TISSUE SECTION(S) WITH ONE TERMINAL NEURITE EXPANSION(TNE), N's IN EACH S WITH MORE THAN ONE TNE, TNE's PER MEISSNER CORPUSCLE(MC), AND PERCENTAGE OF MC's IN EACH S WITH TNE's.

Age(months)	N≥1TNE	N≥>1TNE	TNE/MC	%MC≥TNE
1.5	1.6	0.042	0.50	33
3	6.5	1.1	0.63	62
6	3.5	0.73	0.85	55
9	3.5	0.31	0.70	57
12	2.7	2.4	1.2	68
15	3.5	0.41	0.86	61
18	1.7	0.30	0.74	55
21	1.7	0.35	0.49	39
24	1.7	0.70	0.47	42

TABLE 5

AVERAGE VALUES PER TISSUE SECTION FOR EACH AGE OF MICE FOR THE NUMBER OF MEISSNER CORPUSCLES(MC) WITH NEURITE INTRAEPITHELIAL CONTINUATIONS(NIC), PERCENTAGE OF MC's WITH NIC's, NUMBER OF MC's WITH TERMINAL AXONAL PROCESSES(TAP), AND PERCENTAGE OF MC's WITH TAP's.

Age(months)	MC$\bar{c}$NIC	%MC$\bar{c}$NIC	MC$\bar{c}$TAP	%MC$\bar{c}$TAP
1.5	0.30	7	0.16	2
3	0.64	6	0.60	6
6	0.40	7	0.50	9
9	0.14	2	0.28	2
12	0.47	8	0.80	17
15	0.06	1	0.37	8
18	0.08	2	0.20	5
21	0.083	3	0.28	6
24	0	0	0.40	4

TABLE 6

AREA AND LENGTH AVERAGES OF MEISSNER CORPUSCULAR NEURITES FROM EACH AGE OF MICE FOR VALUES COMPUTED FROM MEASUREMENTS OF CAMERA LUCIDA DRAWINGS WITH A LENZ VIDEOPLAN MICRO COMPUTER.

Age	Area(um^2)	Length(um)
1.5	24	66
3	40	72
6	67	74
9	64	77
12	57	79
15	65	68
18	68	72
21	64	74
24	65	74

TABLE 7

Linear regression statistical results for 17 characteristics of mouse Meissner corpuscles; the independent variable(x) was age in months and the dependent variable was the morphological characteristic quantified.

y	r*	m	b	s
MC/S	-0.946	-0.313043	10.9087	2.48193
B/MC	0.855	0.0387417	0.359753	0.339687
%MC$\bar{c}$B	0.811	1.34651	26.3953	12.4435
MC$\bar{c}$NIC	-0.758	-0.021117	0.498367	0.208788
%MC$\bar{c}$NIC	-0.728	-0.276811	7.34565	2.85259
Area	0.694	1.31884	41.0652	14.2396
N/MC	-0.641	-0.030138	2.13335	0.352766
N$\bar{c}$1TNE	-0.544	-0.1083	4.25099	1.49369
N$\bar{c}$>1B	0.349	0.0188735	1.00371	0.405517
Length	0.251	0.1278	71.334	3.81287
CI/S	-0.202	-0.010046	0.711117	0.372512
%MC$\bar{c}$TNE	-0.155	-0.229578	55.2377	11.0965
MC$\bar{c}$TAP	-0.145	-0.003732	0.444293	0.193589
TNE/MC	-0.142	-0.004196	0.766611	0.220963
N$\bar{c}$>1TNE	-0.047	-0.004205	0.755834	0.666864
%MC$\bar{c}$TAP	0.038	0.022068	6.28706	4.32335
%MC$\bar{c}$CI	-0.300	-0.190547	11.5961	4.75577

*-critical values of r for $H_0: \rho = 0$ at 0.05 and 0.02 confidence limits when d.f.= 7 are 0.667 and 0.750, respectively, for two-tailed test

r= coefficient of linear correlation
m= slope of the regression line
b= y-intercept
s= standard deviation of y

FIGURES

EXPLANATION OF FIGURES 1-3

Photomicrograph of a digital pad section from a mouse 1.5 months old. x250.

Photomicrograph at high magnification of three neurites innervating a Meissner corpuscle from a mouse 1.5 months old. x750.

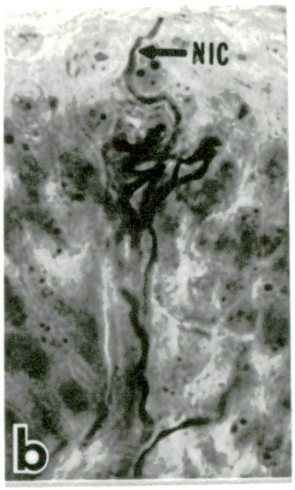
Photomicrograph of a digital pad section from a mouse 3 months old. x250.

Photomicrograph at high magnification of three neurites innervating a Meissner corpuscle from a mouse 3 months old. x750.

Photomicrograph of a digital pad section from a mouse 6 months old. x250.

Photomicrograph at high magnification of a Meissner corpuscular neurite from a mouse 6 months old. x750.


1a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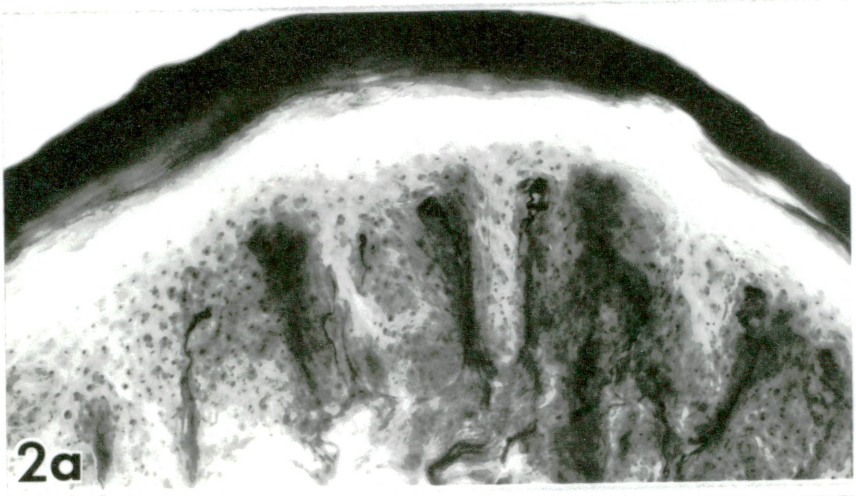
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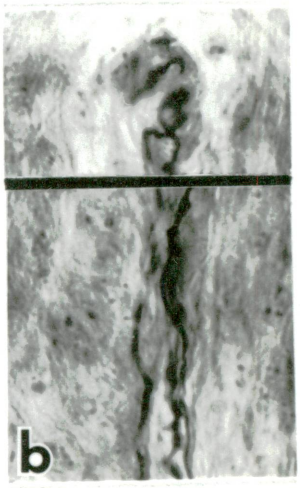
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3a

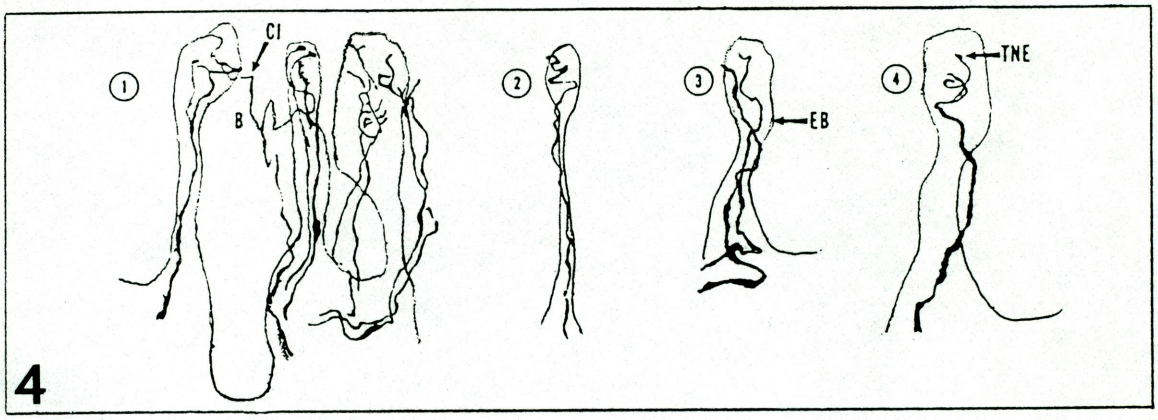

b

EXPLANATION OF FIGURES 4-6

4 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 1.5 months old. x600.

5 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 3 months old. x600.

6 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 6 months old. x600.



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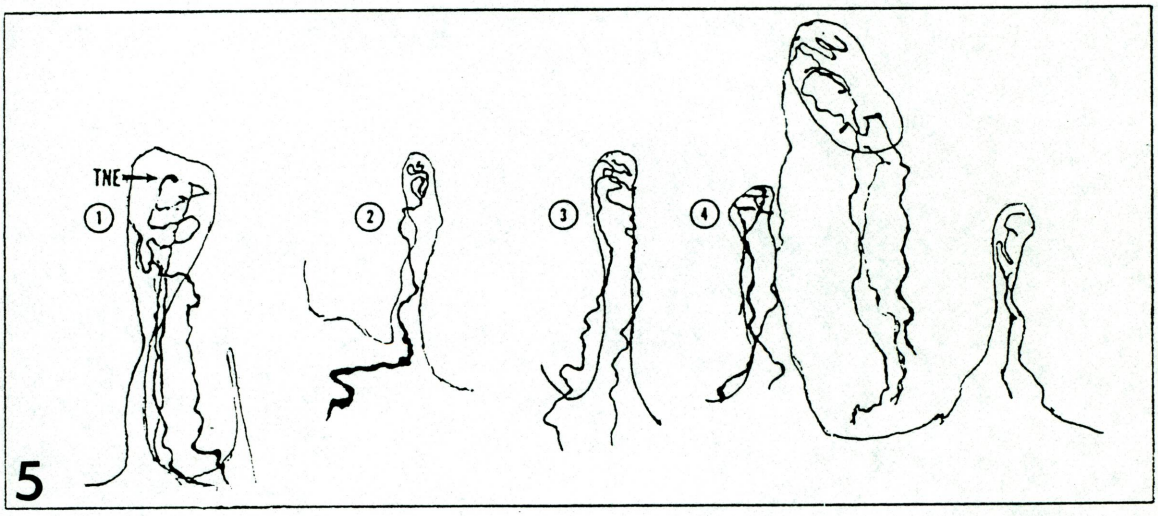

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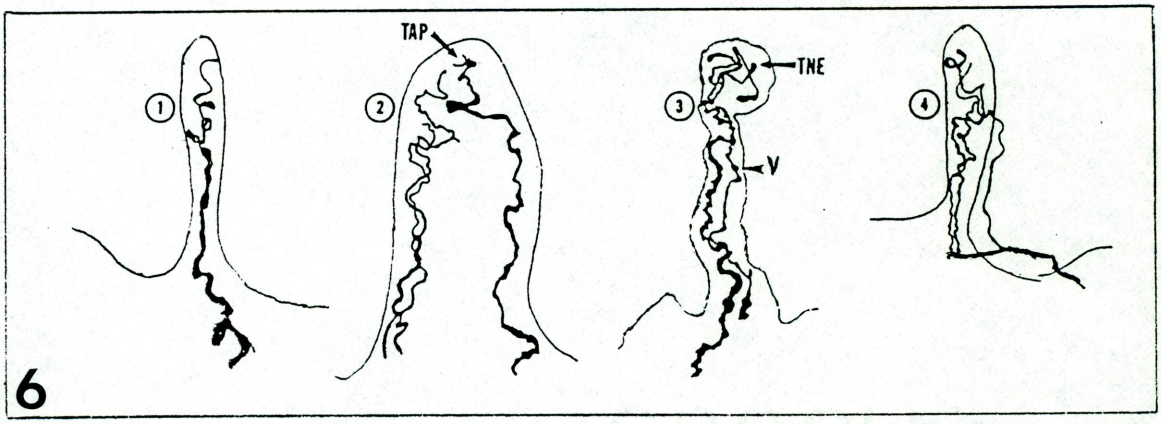


6

EXPLANATION OF FIGURES 7-9

7a Photomicrograph of a digital pad section from a mouse 9 months old. x250.

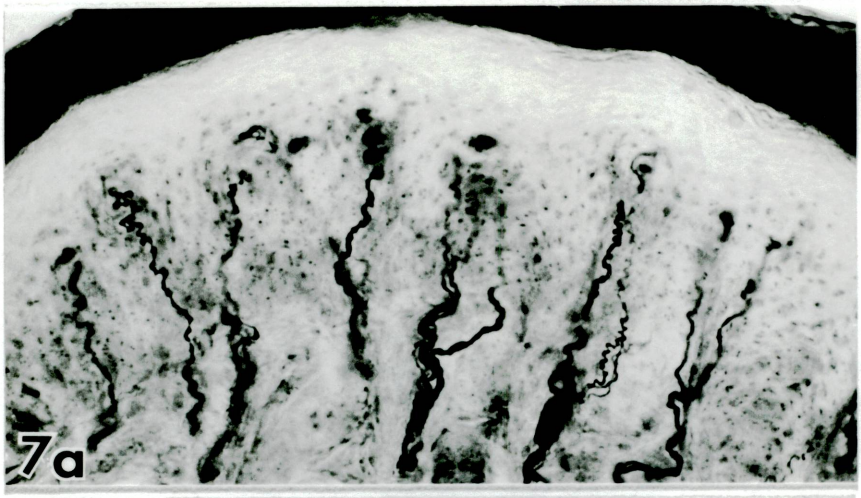
b Photomicrograph at high magnification of two Meissner corpuscular neurites from a mouse 9 months old. x750.

8a Photomicrograph of a digital pad section from a mouse 12 months old. x250.

b Photomicrograph at high magnification of a Meissner corpuscular neurite from a mouse 12 months old. x750.

9a Photomicrograph of a digital pad section from a mouse 15 months old. x250.

b Photomicrograph at high magnification of three neurites innervating a Meissner corpuscle from a mouse 15 months old. x750.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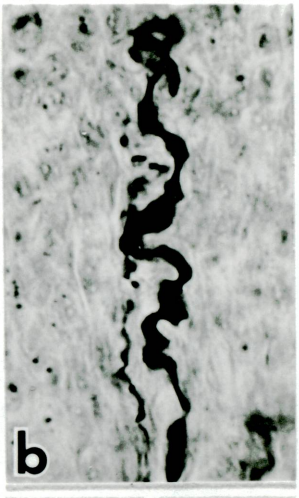
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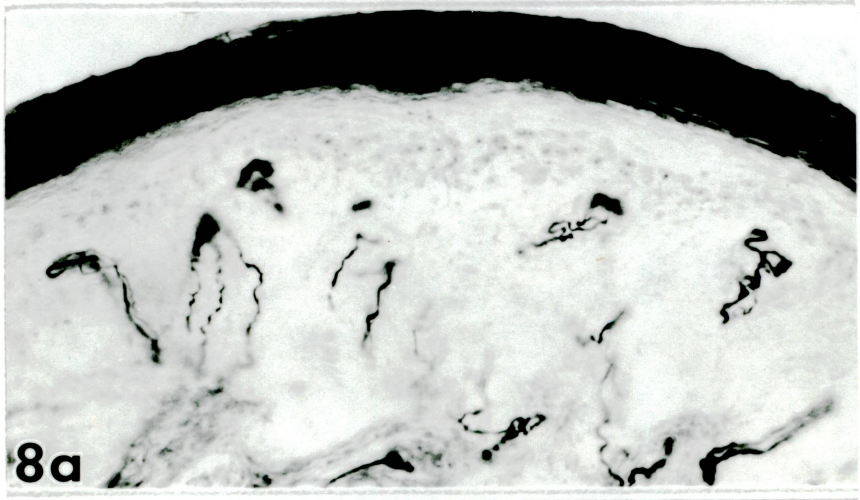
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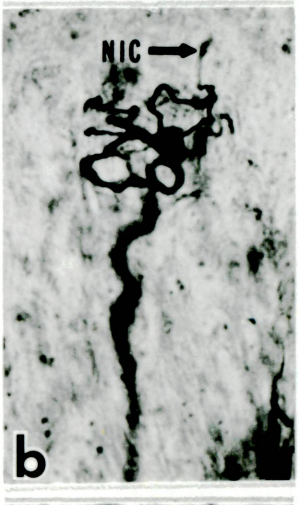
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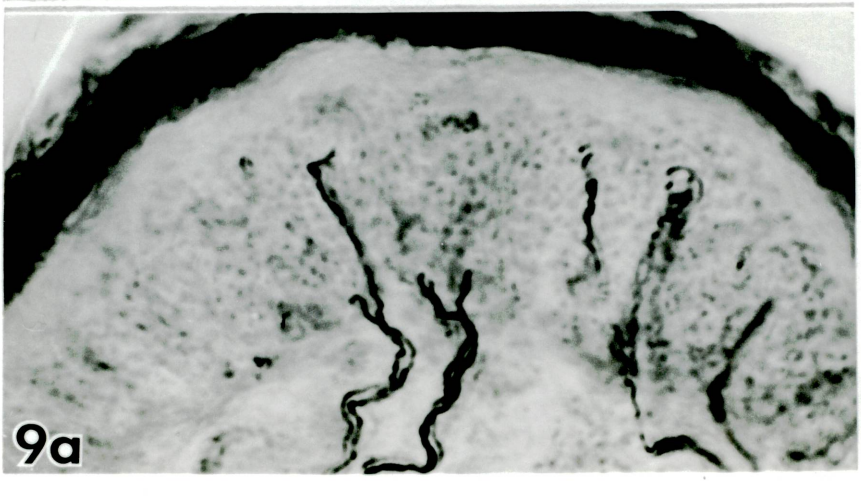
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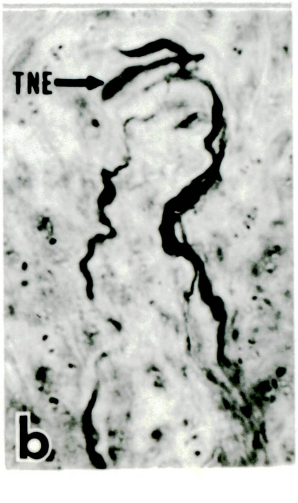
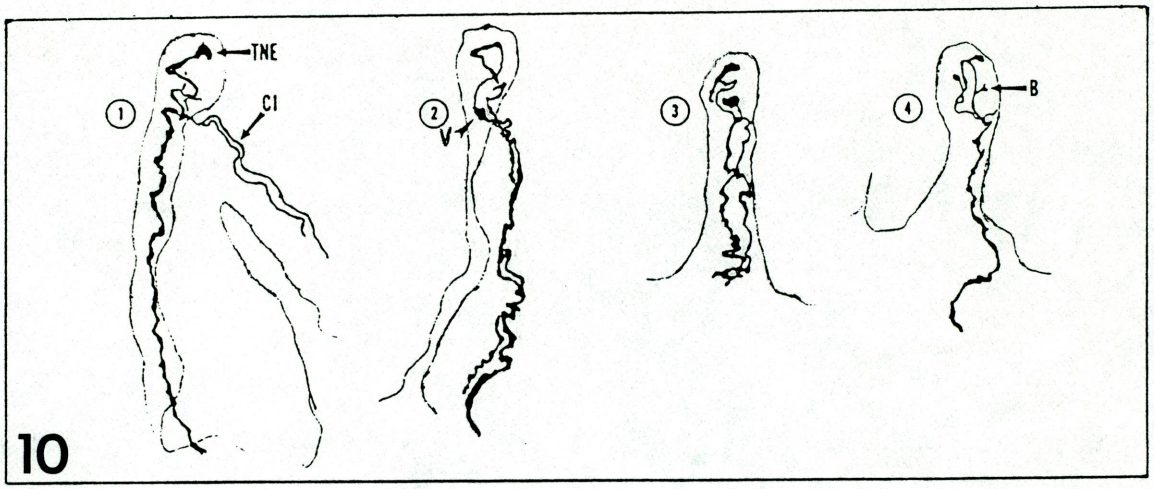

TNE
b

EXPLANATION OF FIGURES 10-12

10 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 9 months old. x600.

11 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 12 months old. x600.

12 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 15 months old. x600.



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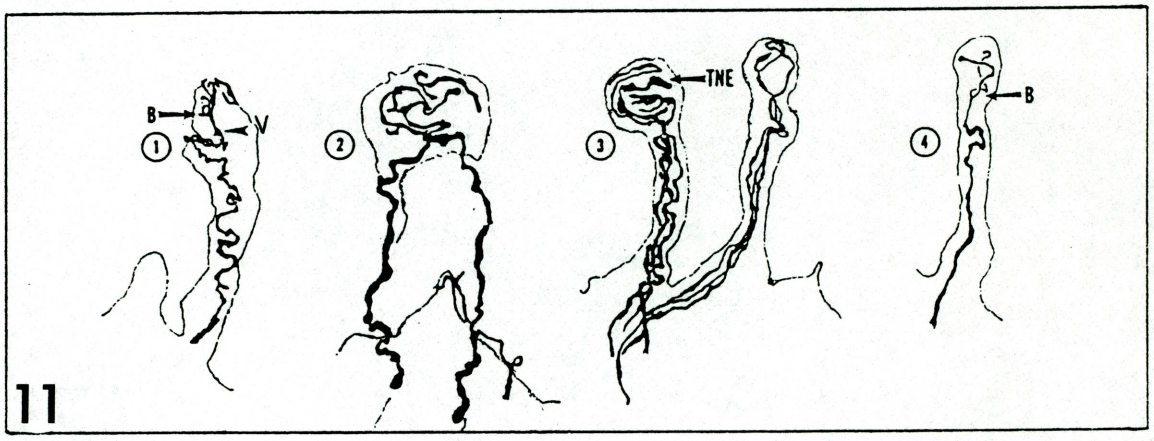

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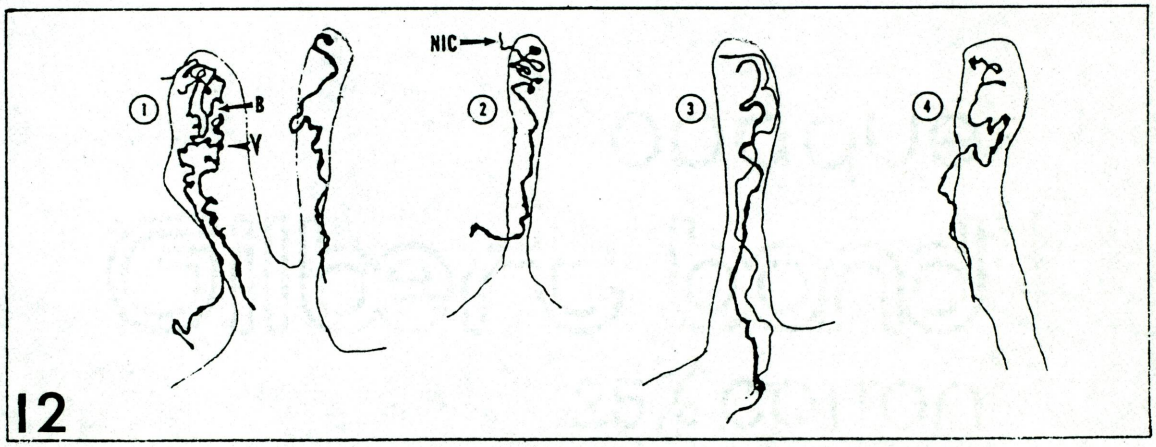


12

EXPLANATION OF FIGURES 13-15

13a Photomicrograph of a digital pad section from a mouse 18 months old. x250.

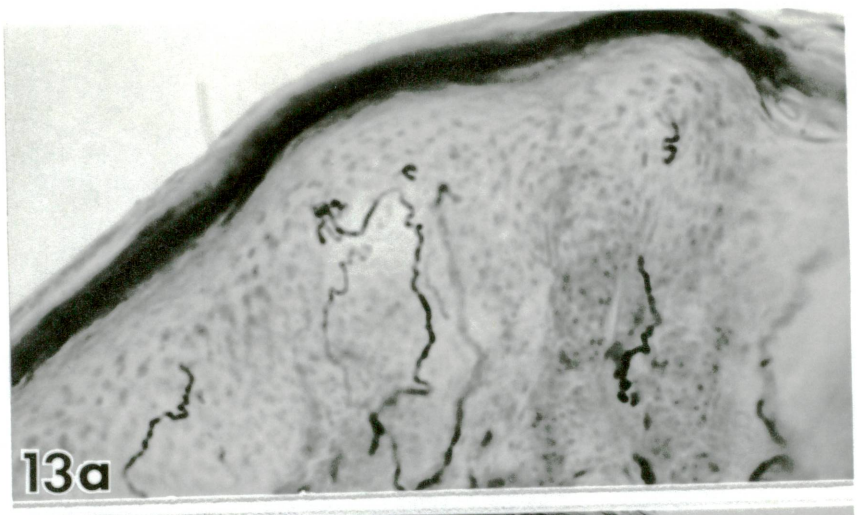
b Photomicrograph at high magnification of a Meissner corpuscular neurite from a mouse 18 months old. x750.

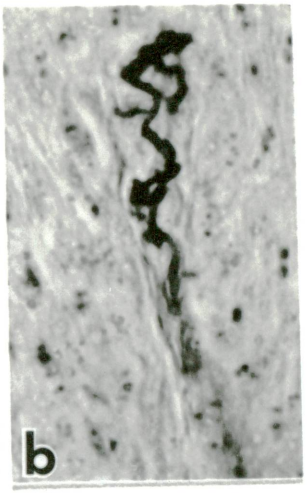
14a Photomicrograph of a digital pad section from a mouse 21 months old. x250.

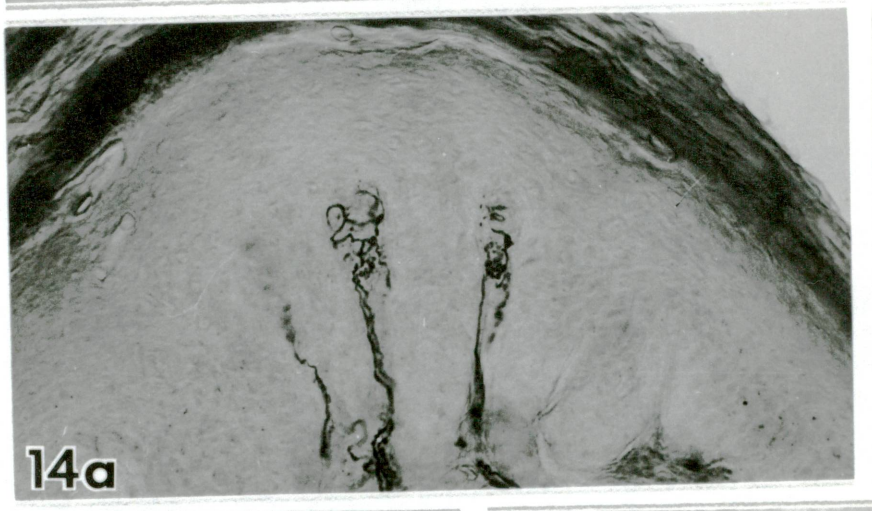
b Photomicrograph at high magnification of two neurites innervating a Meissner corpuscle from a mouse 21 months old. x750.

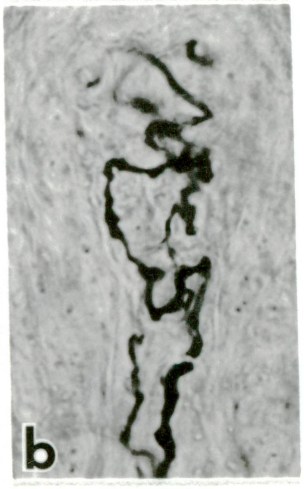
15a Photomicrograph of a digital pad section from a mouse 24 months old. x250.

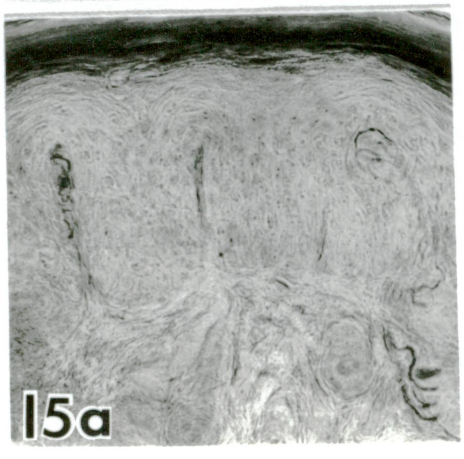
b Photomicrograph at high magnification of a Meissner corpuscular neurite and two empty dermal papillae (arrows). x750.

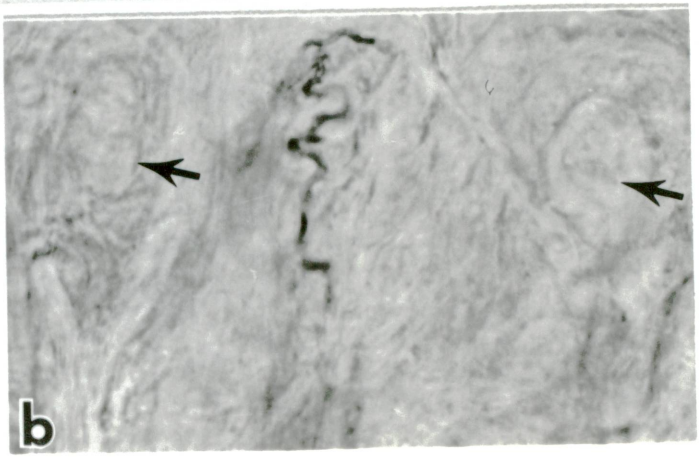

13a


b


14a


b


15a

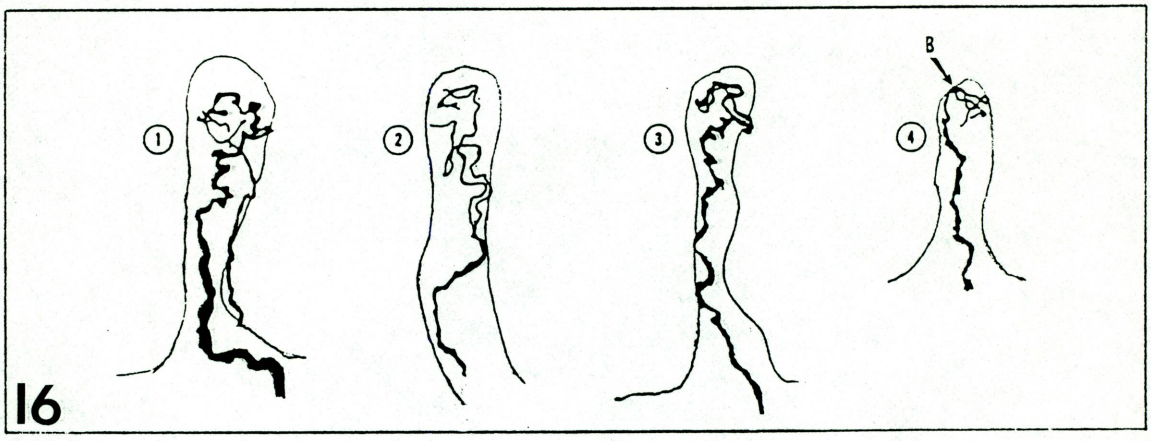

b

EXPLANATION OF FIGURES 16-18

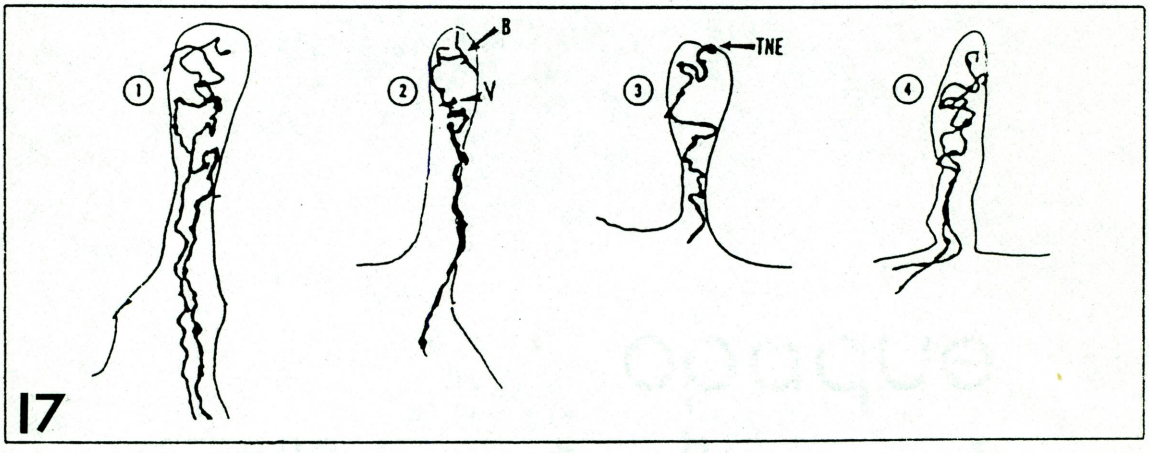
16 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 18 months old. x600.

17 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 21 months old. x600.

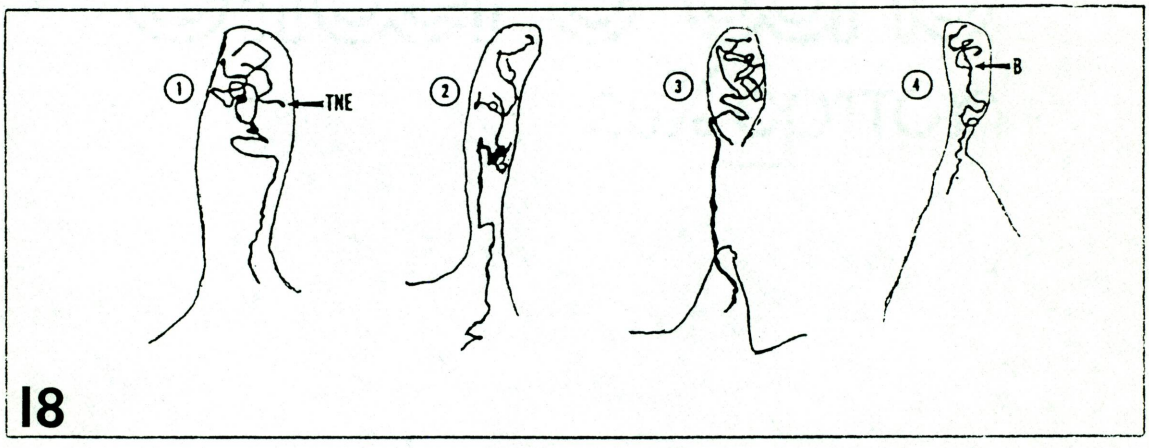
18 Camera lucida tracings illustrating Meissner corpuscular neurites from mice 24 months old. x600.



16



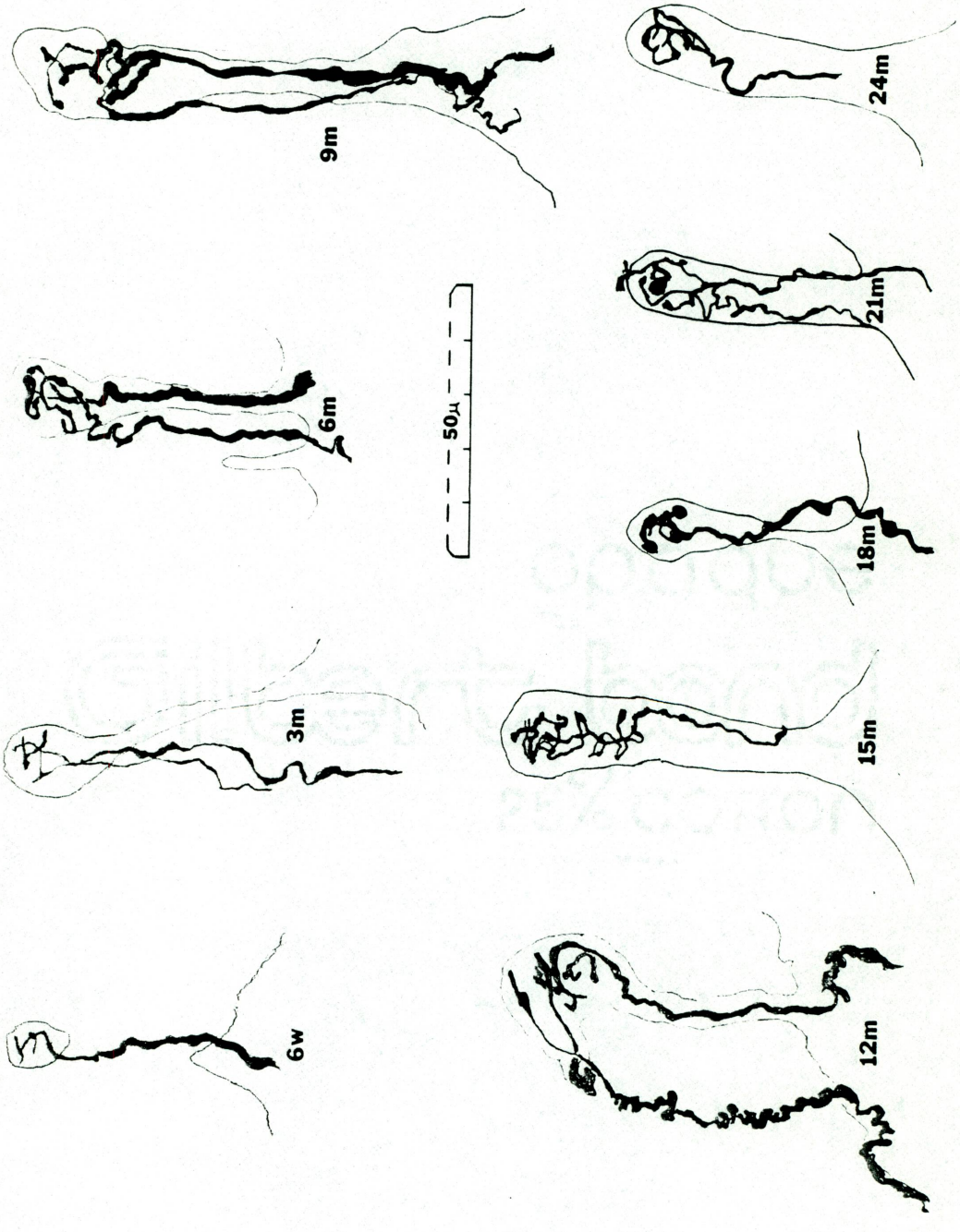
17



18

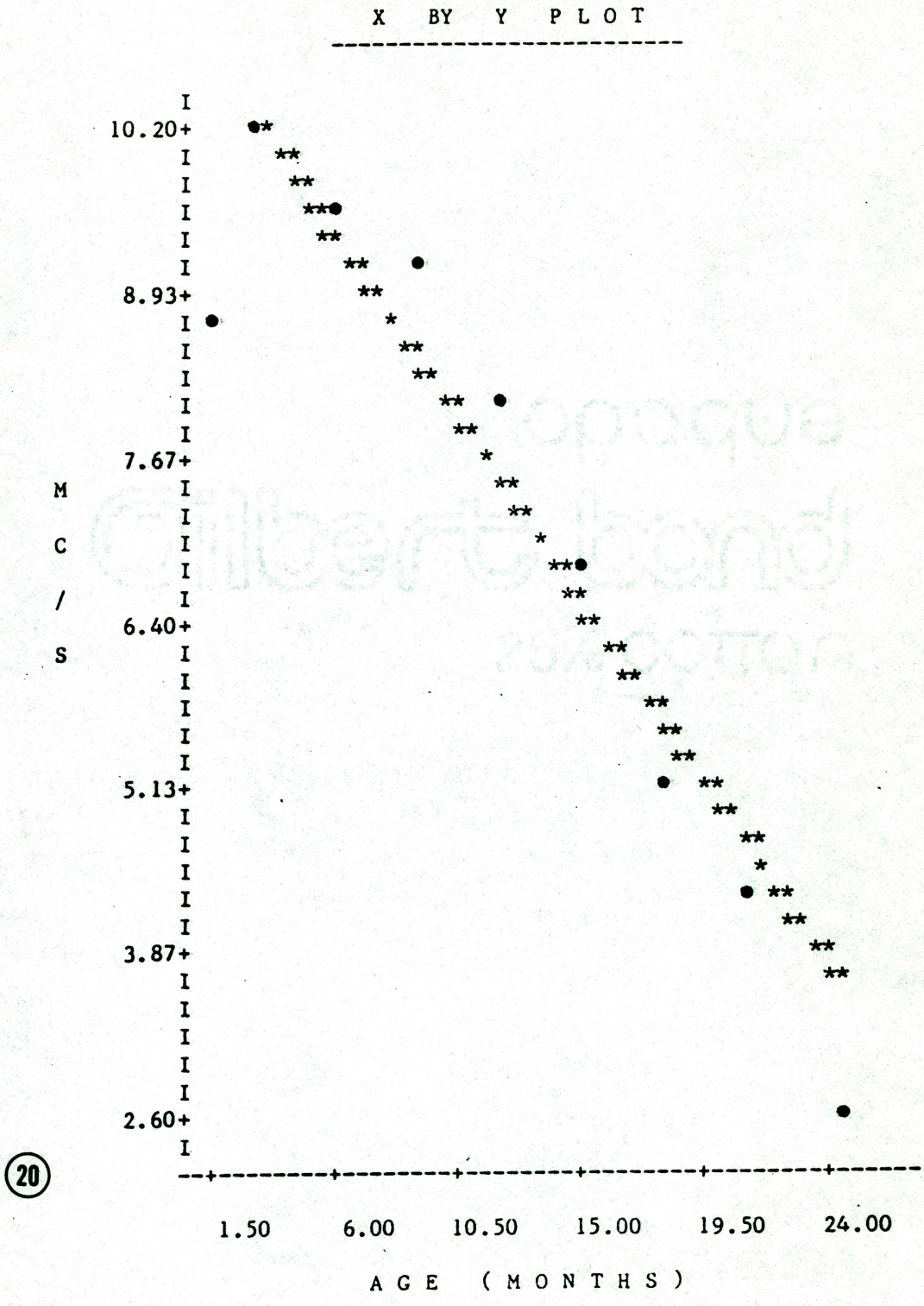
EXPLANATION OF FIGURE 19

Comparison of camera lucida tracings to show age-related size and shape changes of Meissner corpuscular neurites from mice 1.5 to 24 months old. x790.


9m
24m
6m
50μ
21m
18m
3m
15m
6w
12m

EXPLANATION OF FIGURE 20

Graph showing the relationship between age of the mouse and the number of Meissner corpuscles per tissue section (MC/S). Also shown is the line of linear regression.

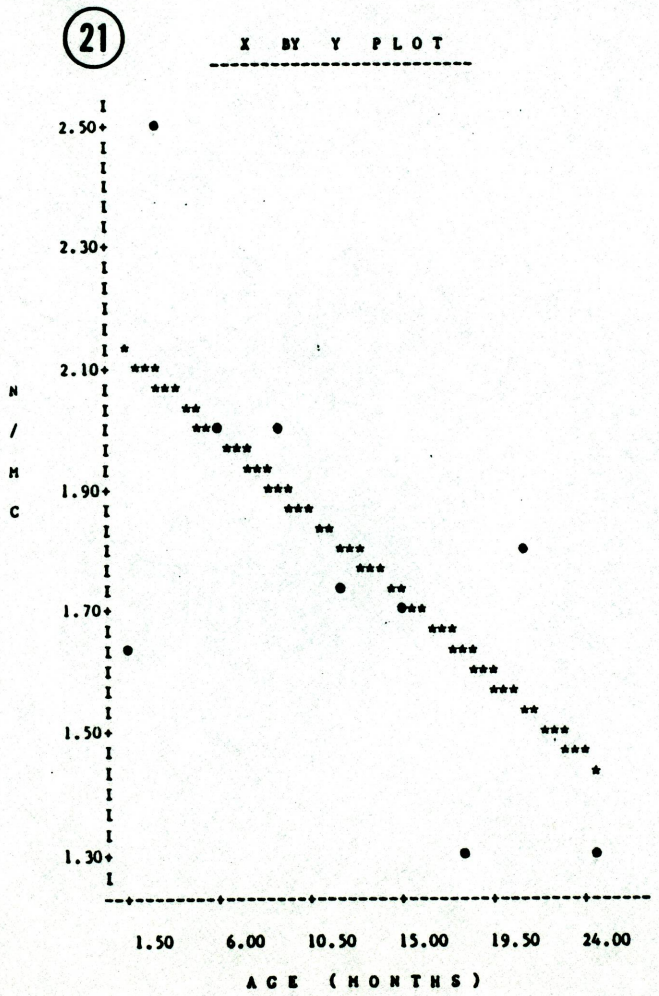

X BY Y PLOT
10.20
8.93
7.67
6.40
5.13
3.87
2.60
M
C
/
S
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

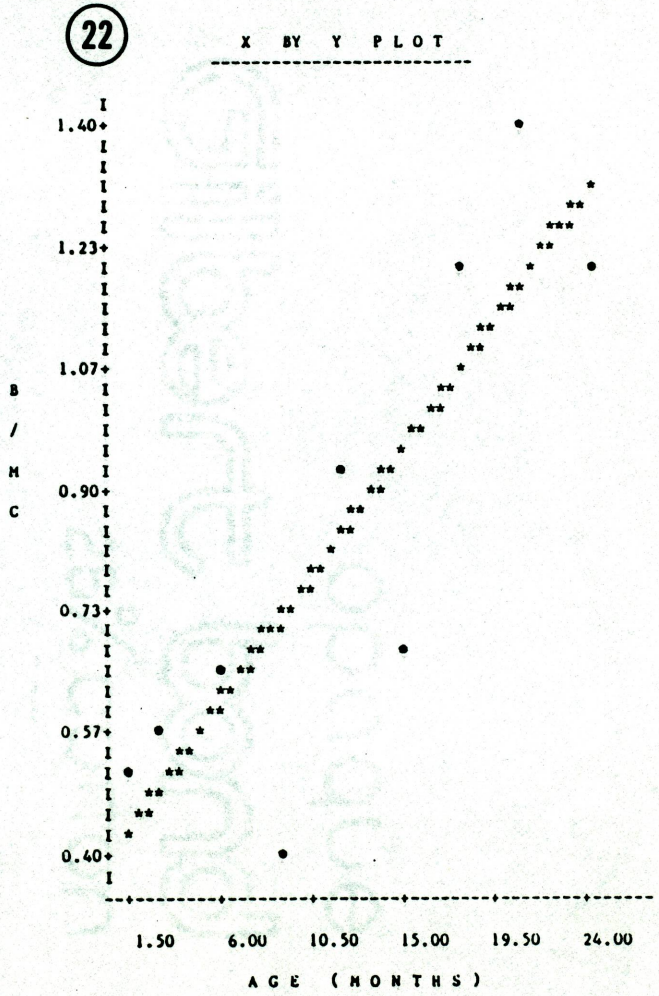
20

EXPLANATION OF FIGURES 21 AND 22

21 Graph showing the relationship between age of the mouse and the number of neurites per Meissner corpuscle (N/MC). Also shown is the line of linear regression.

22 Graph showing the relationship between age of the mouse and the number of branches per Meissner corpuscle (B/MC). Also shown is the line of linear regression.

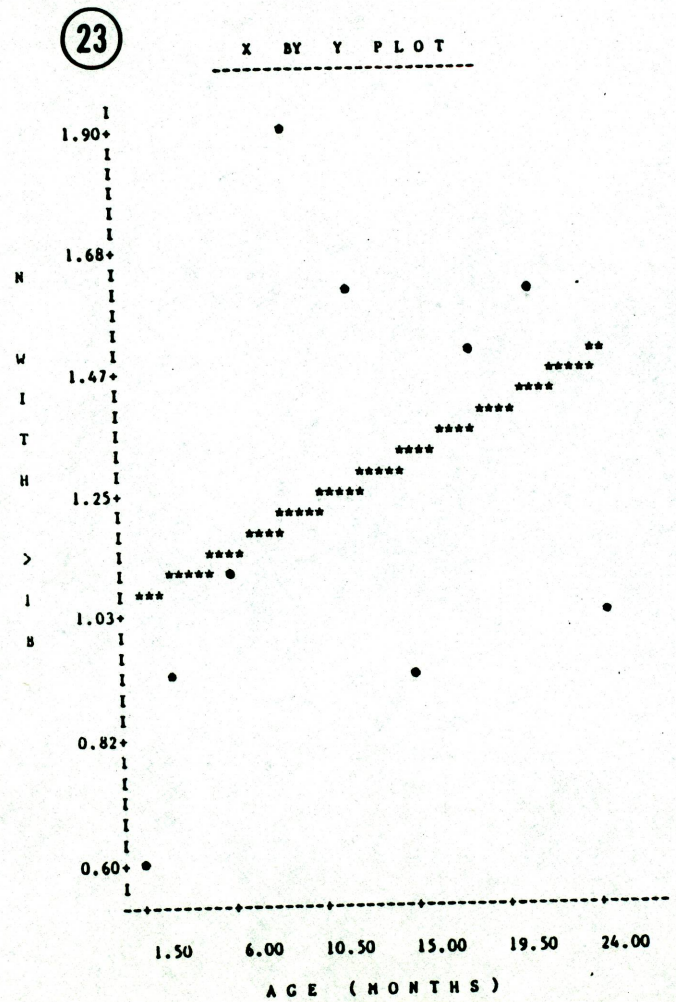

21
X BY Y PLOT
N / M C
2.50
2.30
2.10
1.90
1.70
1.50
1.30
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

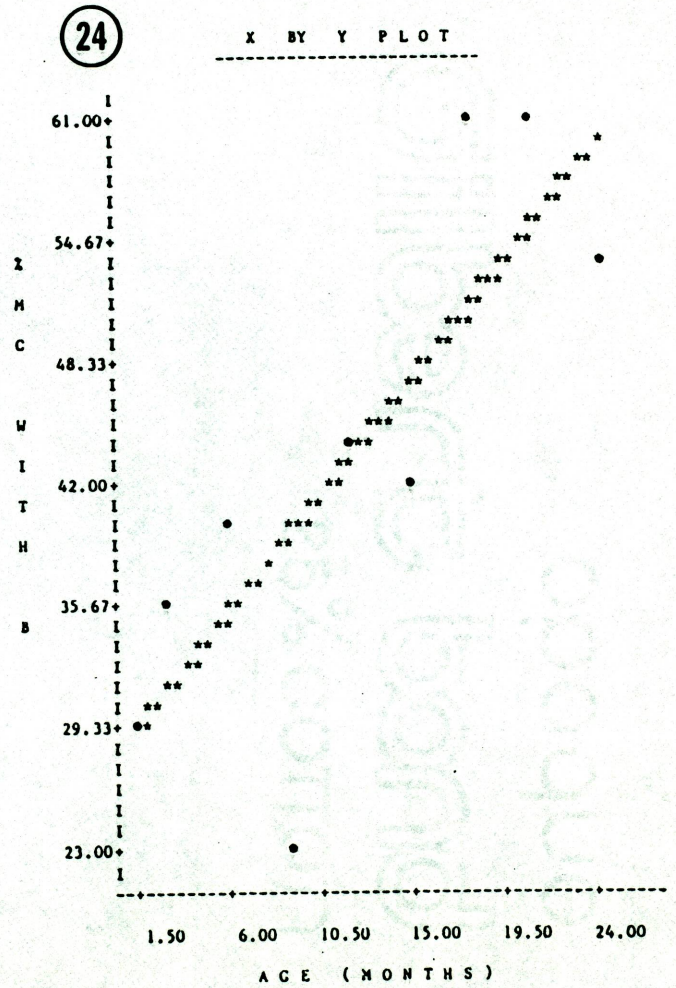

22
X BY Y PLOT
B / M C
1.40
1.23
1.07
0.90
0.73
0.57
0.40
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

EXPLANATION OF FIGURES 23 AND 24

23 Graph showing the relationship between age of the mouse and the number of neurites with more than one branch (N WITH >1B). Also shown is the line of linear regression.

24 Graph showing the relationship between age of the mouse and the percentage of Meissner corpuscles with branching (%MC WITH B). Also shown is the line of linear regression.


23
X BY Y PLOT
1.90
1.68
1.47
1.25
1.03
0.82
0.60
N
W
I
T
H
>
1
8
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

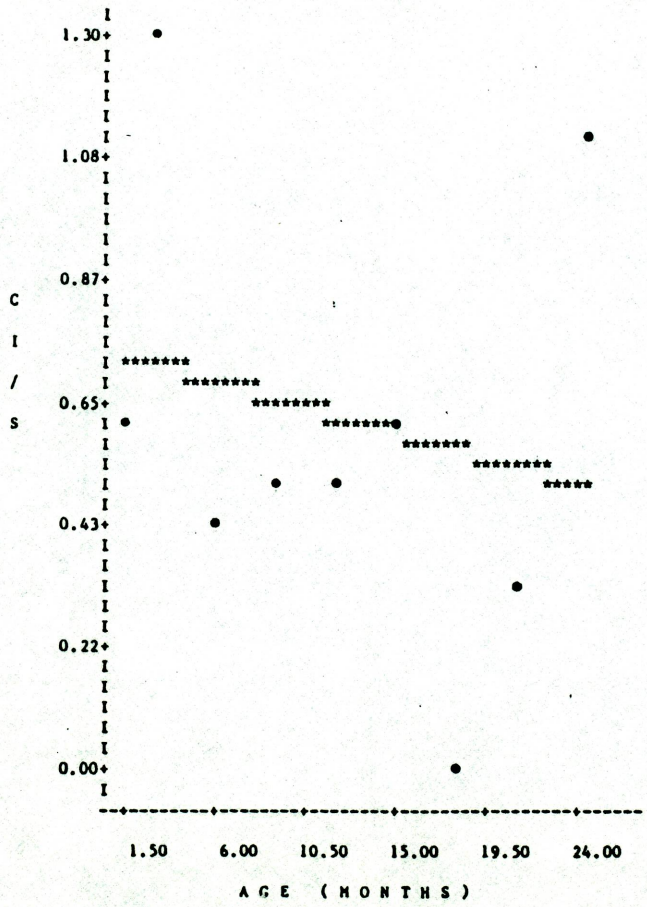

24
X BY Y PLOT
61.00
54.67
48.33
42.00
35.67
29.33
23.00
Z
M
C
W
I
T
H
8
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

EXPLANATION OF FIGURES 25 AND 26

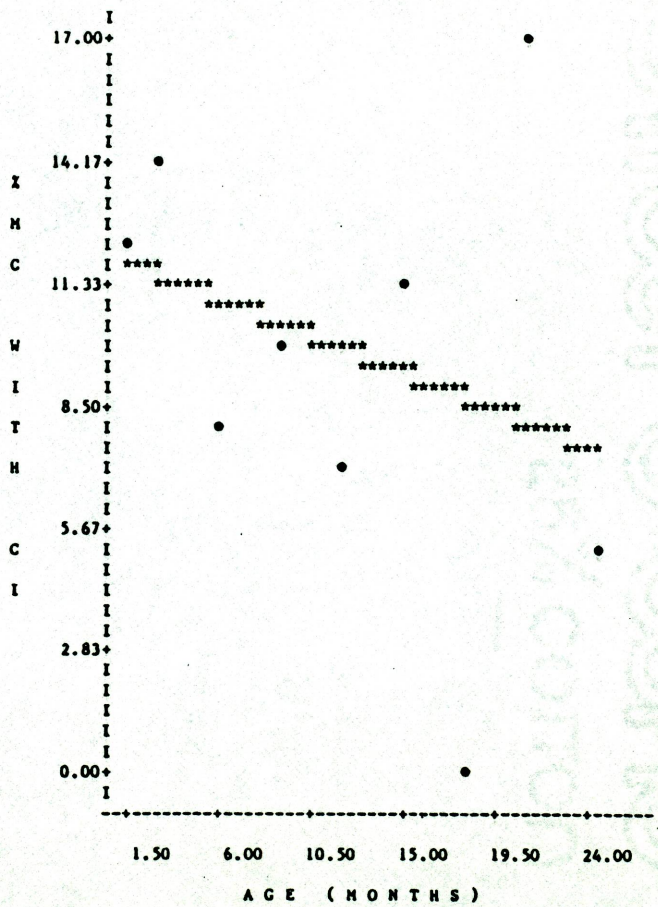
25 Graph showing the relationship between age of the mouse and the number of cross-innervations per tissue section (CI/S). Also shown is the line of linear regression.

26 Graph showing the relationship between age of the mouse and the percentage of Meissner corpuscles involved with cross-innervation (%MC WITH CI). Also shown is the line of linear regression.

25


X BY Y PLOT
1.30
1.08
0.87
0.65
0.43
0.22
0.00
C I / S
1.50 6.00 10.50 15.00 19.50 24.00
AGE (MONTHS)

26


X BY Y PLOT
17.00
14.17
11.33
8.50
5.67
2.83
0.00
X M C W I T H C I
1.50 6.00 10.50 15.00 19.50 24.00
AGE (MONTHS)

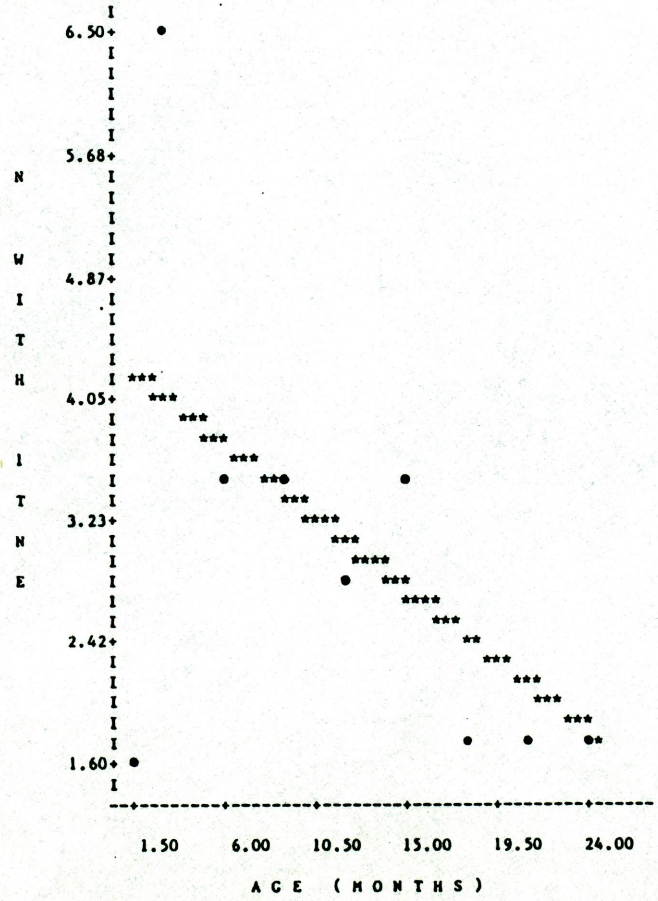
EXPLANATION OF FIGURES 27 AND 28

27 Graph showing the relationship between age of the mouse and the number of neurites in each tissue section with one terminal neurite expansion (N WITH 1TNE). Also shown is the line of linear regression.

28 Graph showing the relationship between age of the mouse and the number of neurites in each tissue section with more than one terminal neurite expansion (N WITH >1TNE). Also shown is the line of linear regression.

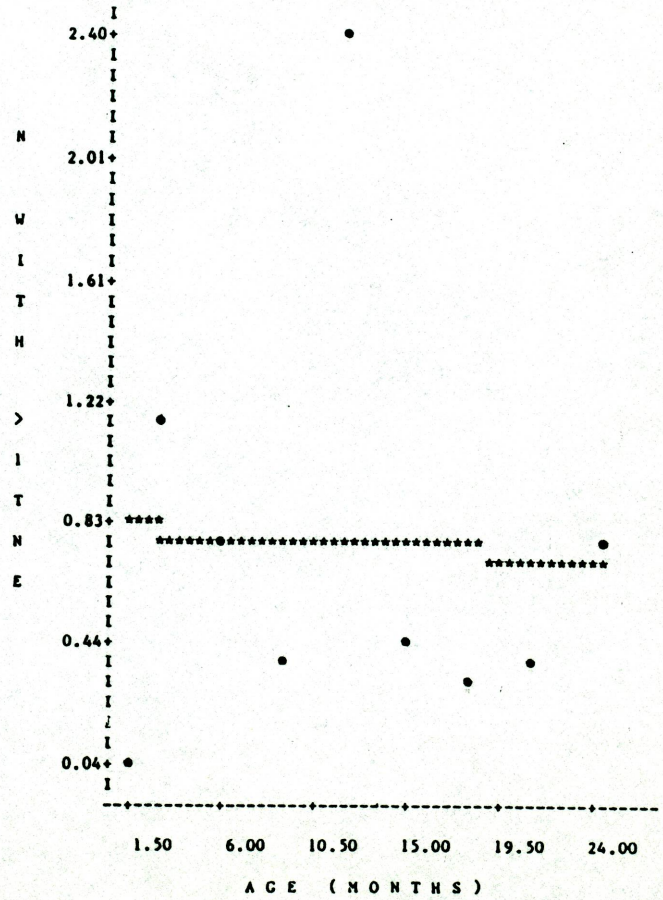
27

X BY Y PLOT



28

X BY Y PLOT



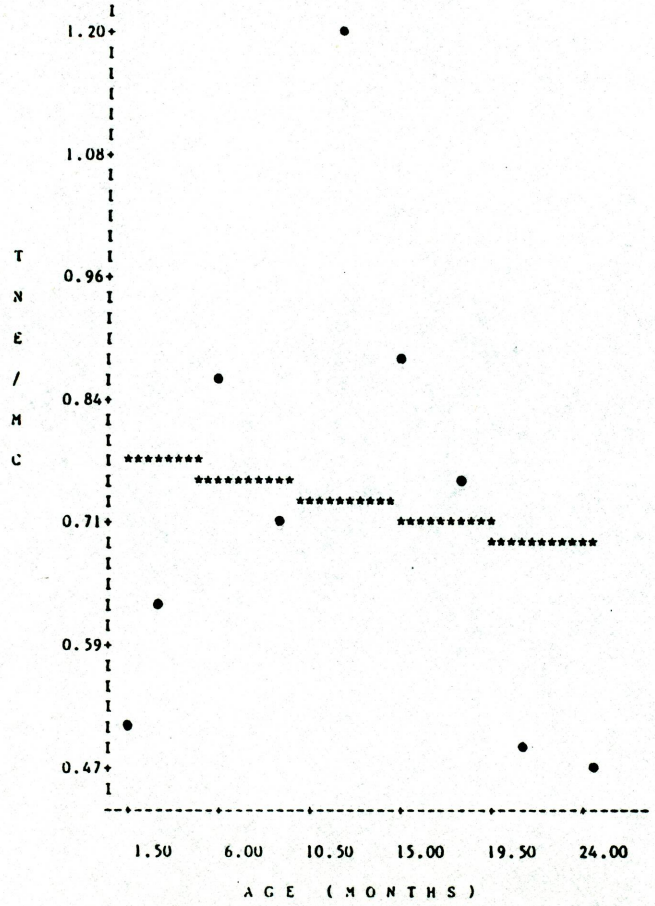
EXPLANATION OF FIGURES 29 AND 30

29 Graph showing the relationship between age of the mouse and number of terminal neurite expansions per Meissner corpuscle (TNE/MC). Also shown is the line of linear regression.

30 Graph showing the relationship between age of the mouse and percentage of Meissner corpuscles with terminal neurite expansions (%MC WITH TNE). Also shown is the line of linear regression.

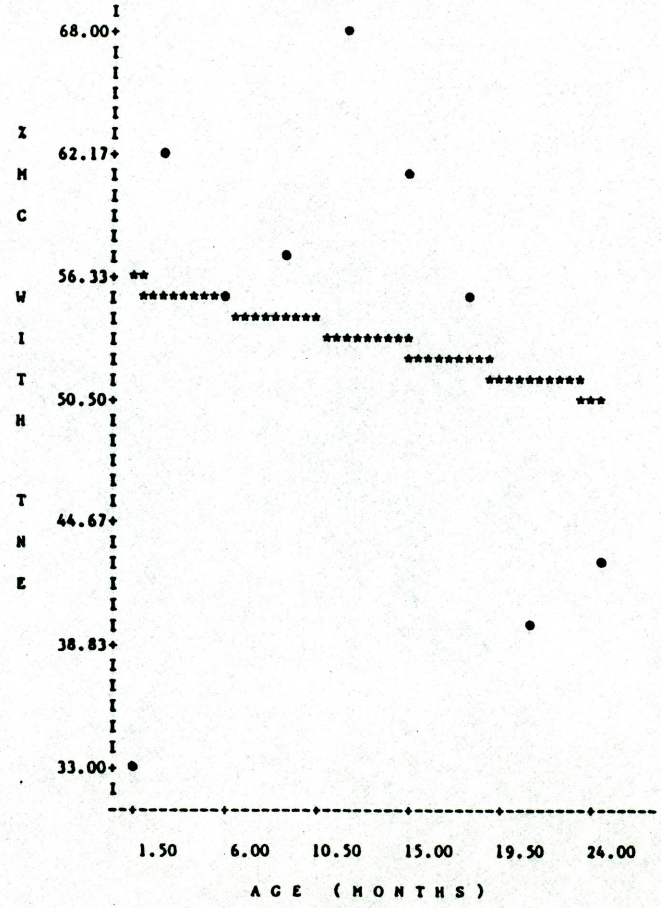
29

X BY Y PLOT



30

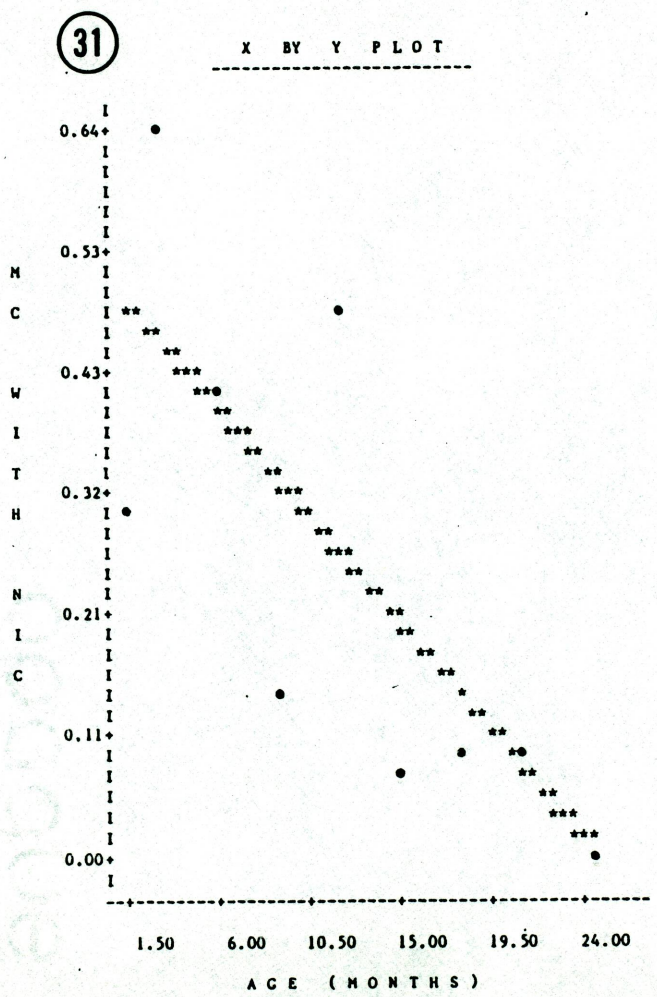
X BY Y PLOT

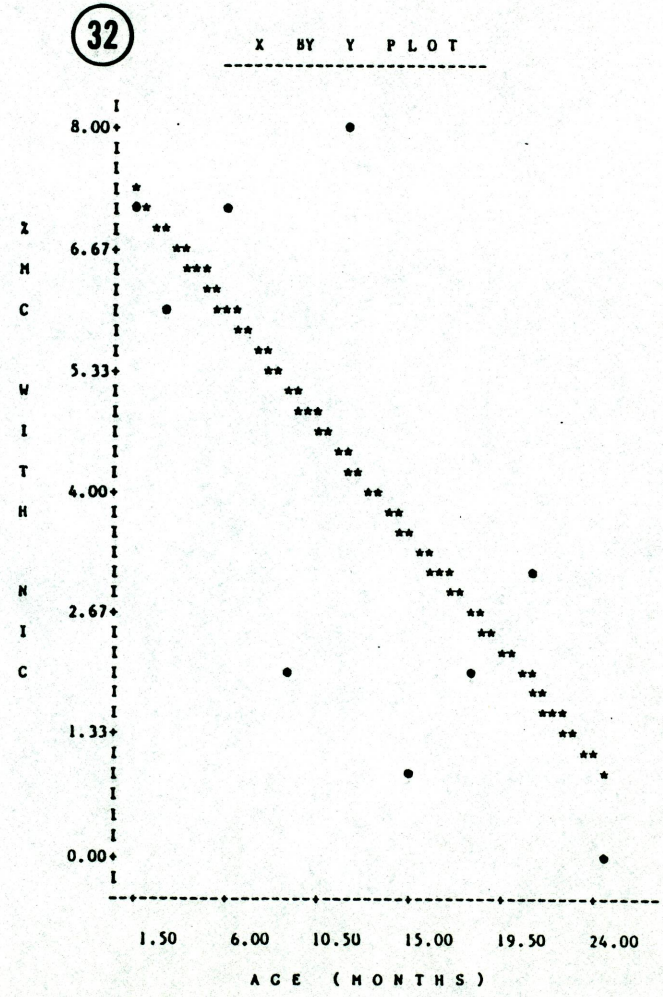


EXPLANATION OF FIGURES 31 AND 32

31 Graph showing the relationship between age of the mouse and number of Meissner corpuscles with neurite intraepidermal continuations (MC WITH NIC). Also shown is the line of linear regression.

32 Graph showing the relationship between age of the mouse and percentage of Meissner corpuscles with neurite intraepidermal continuations (%MC WITH NIC). Also shown is the line of linear regression.


31
X BY Y PLOT
MCG WITH NIC
0.64
0.53
0.43
0.32
0.21
0.11
0.00
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)


32
X BY Y PLOT
MCG WITH NIC
8.00
6.67
5.33
4.00
2.67
1.33
0.00
1.50
6.00
10.50
15.00
19.50
24.00
AGE (MONTHS)

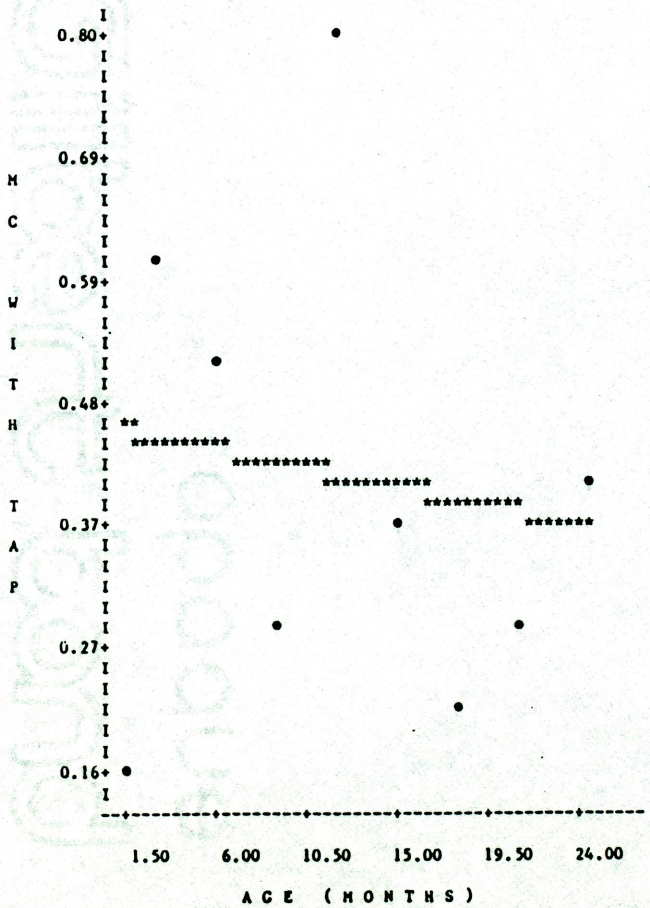
EXPLANATION OF FIGURES 33 AND 34

33 Graph showing the relationship between age of the mouse and number of Meissner corpuscles with terminal axonal processes (MC WITH TAP). Also shown is the line of linear regression.

34 Graph showing the relationship between age of the mouse and percentage of Meissner corpuscles with terminal axonal processes (%MC WITH TAP). Also shown is the line of linear regression.

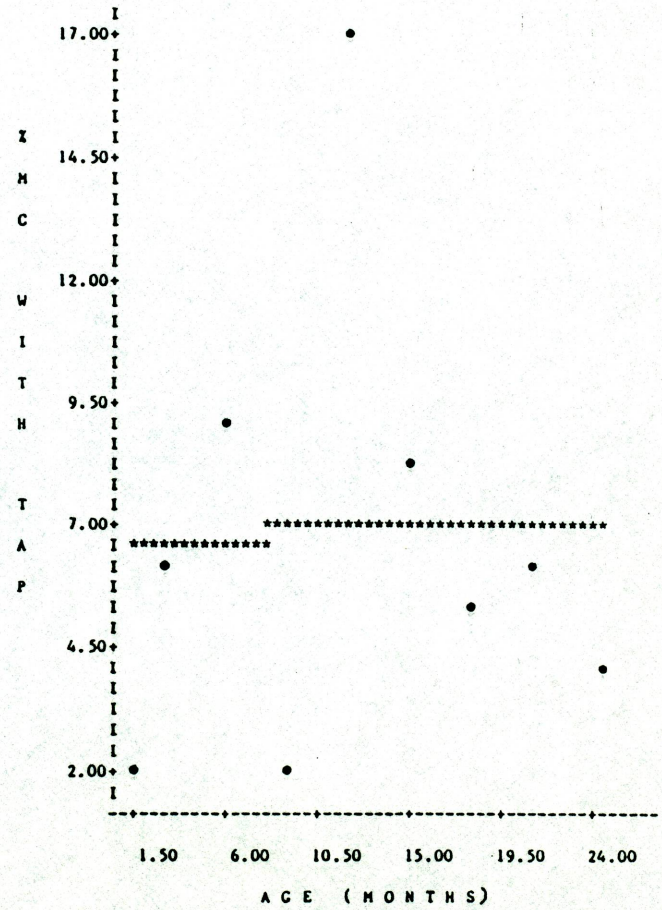
33

X BY Y PLOT



34

X BY Y PLOT



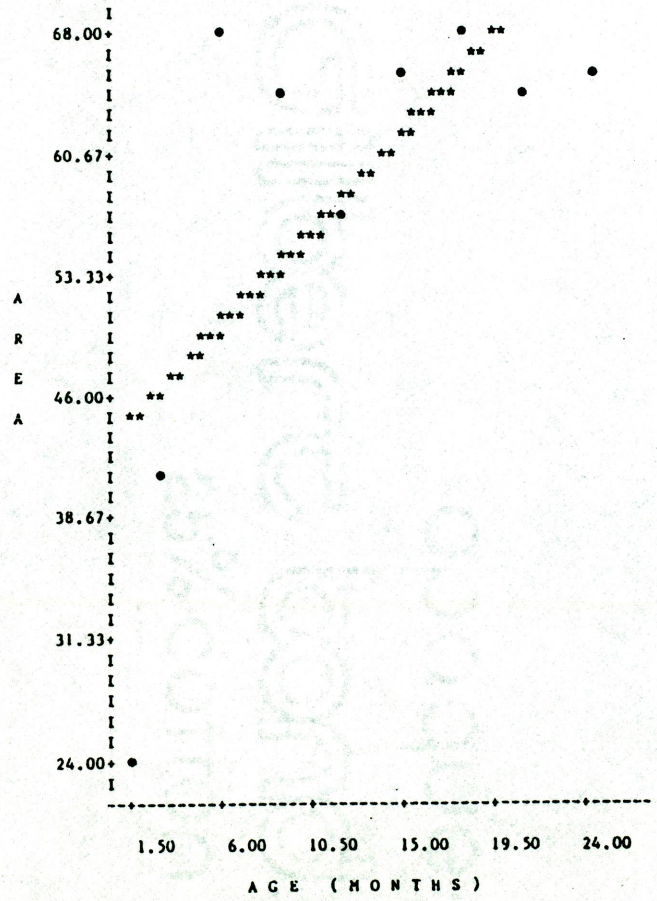
EXPLANATION OF FIGURES 35 AND 36

35 Graph showing the relationship between age of the mouse and area of the neurite. Also shown is the line of linear regression.

36 Graph showing the relationship between age of the mouse and length of the neurite. Also shown is the line of linear regression.

35

X BY Y PLOT



36

X BY Y PLOT