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Qualitative structural development of the feline inferior alveolar nerve

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INTRODUCTION

During peripheral nerve development, the initial outgrowth of pioneer axons and the appearance of Schwann cell precursors is followed by a sequence of maturational steps including both growth in size and structural differentiation (reviewed by Webster, 1975; Landon & Hall, 1976; Ochoa, 1976; Berthold, 1978). Studies on hind limb nerves in young kittens have shown that, in axons destined to become large, completion of the structural differentiation coincides in time with attainment of almost mature functional properties. This takes place at an axonal diameter far below the final size (Berthold, 1968; Schwieler, 1968; Skoglund, 1969; Berthold, 1978). The functional development of cutaneous, articular and muscular afferent units in kitten hind limbs also seems to occur in orderly relation to the changing properties of the afferent nerve fibres (Ekholm, 1967; Skoglund, 1969). In this context, an understanding of nerve fibre maturation in trigeminal branches related to the teeth would be of interest, particularly in view of the extensive developmental remodelling of the dentition. The cat inferior alveolar nerve appears suitable for examination since it enters the mandibular canal as a distinct trunk (cf. Thomas, 1946) and the distribution of its branches has been described (Robinson, 1979).

According to light microscopic data, some 50% of the axons of the inferior alveolar nerve enter the mental nerves and convey sensory information from the chin and lower lip (Thomas, 1946; Robinson, 1979). The other axons of the nerve transmit impulses from the pulps of the mandibular teeth as well as from the associated periodontal ligaments and gingiva (Bannister, 1976; Robinson, 1979). In addition, some sympathetic axons seem to course within the inferior alveolar nerve (Christensen, 1940; Matthews & Robinson, 1980). With respect to the development of the feline inferior alveolar nerve, no information is available, apart from a quantitative light microscopic study (Mohiuddin, 1951). In the present work, we have examined this nerve by electron microscopy from the fetal period throughout development to the fully mature and old adult stages. The principal aim has been to establish a chronology for the qualitative structural development of this nerve.

MATERIAL AND METHODS

The material was taken from 56 fetal and postnatal kittens and 21 adult cats, ranging in age from 25 days post conception (dpc) to 11 years after birth. The gestational ages of the prenatal kittens were counted from 24 hours after parental mating (Stein, 1975). The relation between crown–rump length, weight and estimated fetal age agreed with previous data (Windle & Griffin, 1931; Coronios, 1933). The average

gestational age of the newborn kittens was 67 ± 5 days (cf. Coronios, 1933; Stein, 1975). Animals aged 7 months were regarded as adults (cf. Shapiro, 1930; Stein, 1975).

The prenatal kittens were delivered by Caesarean section while the mother was anaesthetized with Halothane®. The fetus aged 25 dpc was fixed by immersion *in toto*. The older fetuses were perfused through the heart with Tyrode's solution followed by 5 % glutaraldehyde in a 300 mOsm phosphate buffer. The perfusates were at room temperature and were infused at constant pressure. The postnatal kittens and the cats were anaesthetized with Mebumal® (40 mg/kg, i.p.), tracheostomised and artificially ventilated with air. After a thoracotomy, they were perfused through the ascending aorta with Tyrode's solution (+37 °C) containing 2·7 % Dextran (26 g T70 and 1 g T500) (Pharmacia, Sweden), followed first by warm (+37 °C) and then by cold (+4 °C) purified 5 % glutaraldehyde in a 300 mOsm phosphate buffer with 2·7 % Dextran (Karlsson & Schultz, 1965; Berthold, 1968). After perfusion the mandible was removed, post-fixed in chilled fixative (4 hours) and stored overnight in cold buffer. In the prenatal kittens aged 25–40 dpc entire mandible halves were processed as described below. The inferior alveolar nerve was later trimmed out by taking semithin transverse sections through the mandibular canal close to the mandibular foramen. In older prenatal kittens and postnatal animals, specimens were taken from the inferior alveolar nerve at its entrance in the mandibular canal. The specimens were osmicated (4 hours) in 2 % OsO_4 in phosphate buffer, rinsed in the buffer, dehydrated in acetone and embedded in Vestopal W (Ryter & Kellenberger, 1958; Berthold, 1968). Thin (silver-grey) transverse and longitudinal sections were cut with an LKB Ultrotome III 8800. The thin sections were collected on one-hole copper grids coated with carbon stabilised formvar, contrasted with uranyl acetate and lead citrate and examined in a Philips EM 300 or 301 electron microscope.

RESULTS

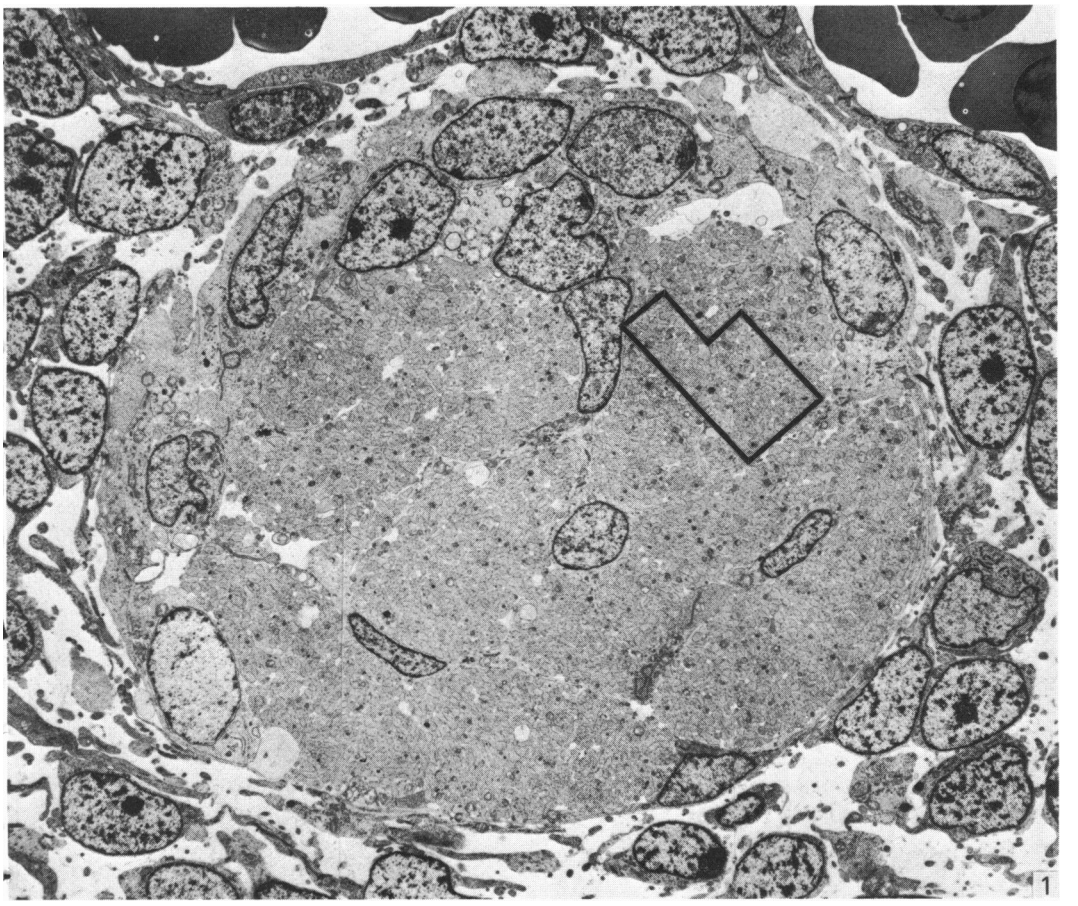
25 *dpc*

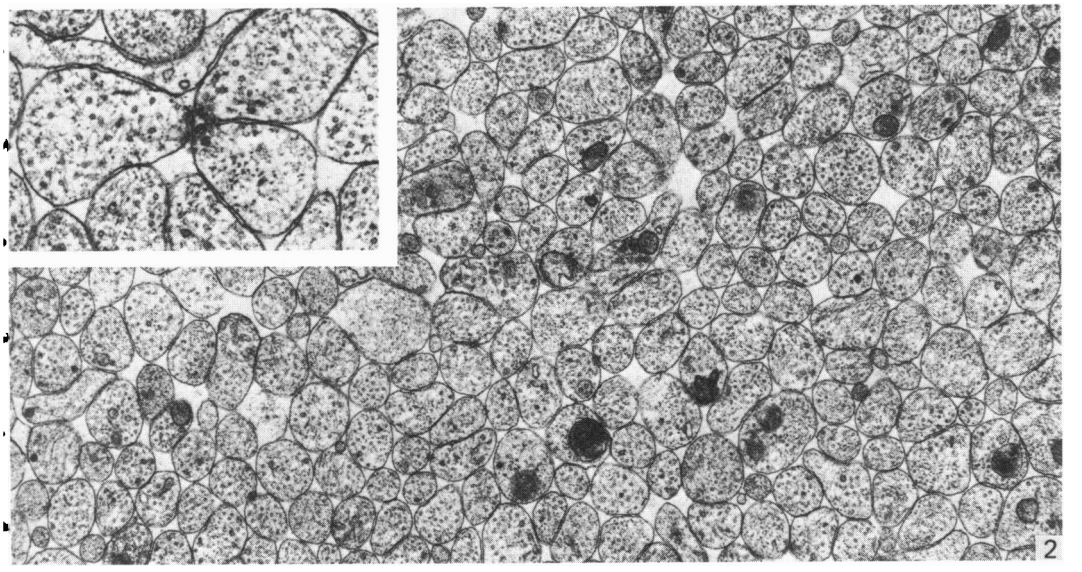
At this stage an inferior alveolar nerve was present in the mandible process, and was composed of small, densely packed unmyelinated axons (Figs. 1, 2). Along the circumference of the nerve a layer of cells formed a largely complete investment (Fig. 1). Cytoplasmic processes from these cells followed the circumference or penetrated into the nerve, and similar cells extended from the circumferential layer into the core of the axon bundle (Fig. 1). These cells lacked distinct basement membranes and did not individually ensheath single axons or groups of axons. Both circumferential and interstitial processes were extensively linked through specialized junctions.

Figs. 1–14. Electron micrographs from the developing, mature and ageing inferior alveolar nerve. Cross sections are shown, unless stated otherwise.

Fig. 1. 25 dpc. General survey of primitive axons and sheath cells. Blood vessels (upper corners) and undifferentiated cells surround the nerve. × 3000. Framed region is shown at a larger magnification in Fig. 2.

Fig. 2. 25 dpc. Detail of Fig. 1, illustrating the densely packed axons of the nerve. × 19300. Inset: Accumulation of electron-dense material at sites of axonal contact. × 36600.





35 dpc

Apart from a greater size range, the axons were generally similar to those present at 25 dpc (Fig. 3). A few axonal profiles, however, had expansions containing large irregular vesicles (Fig. 4) and others contained clusters of small rounded vesicles (Fig. 5) or dense lamellated bodies. Similar formations have been interpreted as tips of growing axons by other workers (see del Cerro & Snider, 1968; Tennyson, 1970). Most of the axons were collectively ensheathed in bundles by interstitial and circumferential cells, which were in some ways similar to those seen at 25 dpc (Fig. 3). A few large axons occupied separate cytoplasmic pockets in the cells (Fig. 4). Distinct basement membranes covered regions of the plasma membrane facing other cells, and at such sites bundles of collagen fibrils were present (Fig. 4). These cells therefore appear to be immature Schwann cells (cf. Webster, 1975). The nerve was completely invested by a few layers of flattened cells containing glycogen granules and possessing long circumferential processes. This sheath resembled a primitive perineurium, but few pinocytotic vesicles were observed, and the cellular layers lacked basement membrane investments.

40 *dpc–birth*

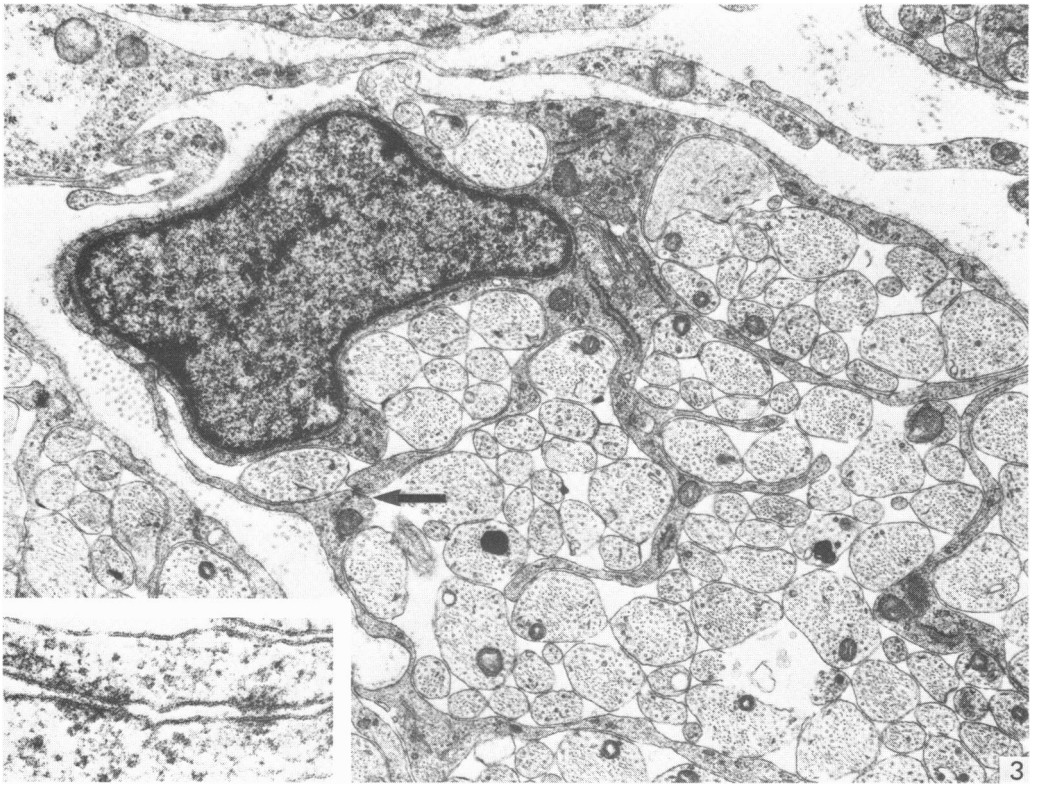
At 40–45 dpc the most striking change in the inferior alveolar nerve was a marked numerical increase and further differentiation of the Schwann cells. All axons were individually ensheathed by Schwann cells. Several profiles resembling unmyelinated axons appeared swollen and electron-lucent (Fig. 6). Single larger (about 0·8 μm) unmyelinated axons ensheathed by Schwann cells in a 1:1 relation were rather common, and some of these showed typical promyelin features (Friede & Samorajski, 1968). At 45 dpc, many larger (about 1 μm) axons were surrounded by a few layers of compact myelin. With the onset of myelination, blood vessels, collagen fibrils and fibroblasts (cf. Bischoff, 1970) appeared in the endoneurium, which was delimited by an immature perineurium.

During the last two prenatal weeks, myelination continued rapidly. Some myelin sheaths related to larger (2–3 μm) axons appeared very irregular and partly fragmented in transverse sections (Fig. 7). In longitudinal sections, these constellations corresponded to short (15–20 μm) and highly distorted internodes, bordered by nodes of Ranvier (Fig. 8). Some similarly sized axon profiles had internodes composed only of Schwann cell cytoplasm loaded with myelin fragments, which was intercalated between the paranodal segments of adjacent intact myelin sheaths (Fig. 9). Macrophages (Gibson, 1979; Oldfors, 1980) containing myelin debris were sometimes associated with such Schwann cells (Fig. 10). Defects in the basement membrane of the Schwann cell were observed at points of close contact between cells (Fig. 11). At birth, separation and myelination appeared to continue, and the perineurium of the nerve was then almost mature (Low, 1976).

Fig. 3. 35 dpc. Immature Schwann cell. Some of its extensively branching processes enclosing bundles of axons can be seen. Regions of the plasma membranes not facing axons are apposed by a basement membrane and collagen fibres. × 16500. Specialized junctions between immature Schwann cell processes (arrow) are shown at a high magnification in the inset. × 74800.

Fig. 4. 35 dpc. This Figure shows an axon profile (*a1*) with an expansion containing irregular vesicles. Another axon (*a2*) occupies a separate Schwann cell pocket. A basement membrane covers the outer Schwann cell surface. × 28900.

Fig. 5. 35 dpc. Partly ensheathed profile (*a*) containing a cluster of rounded vesicles. × 46600.



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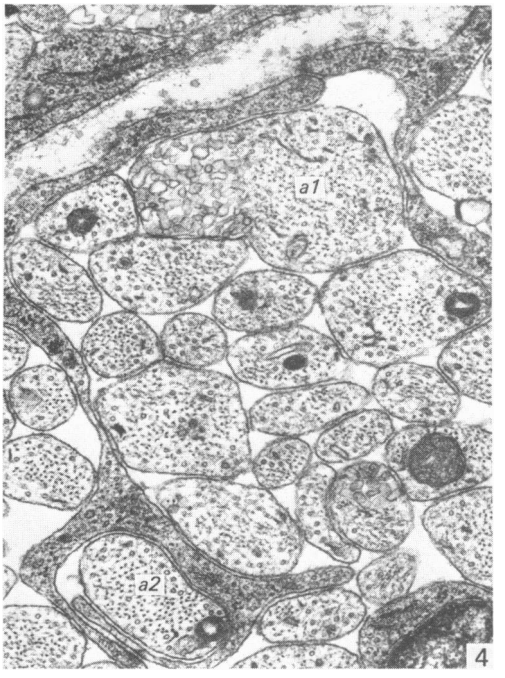

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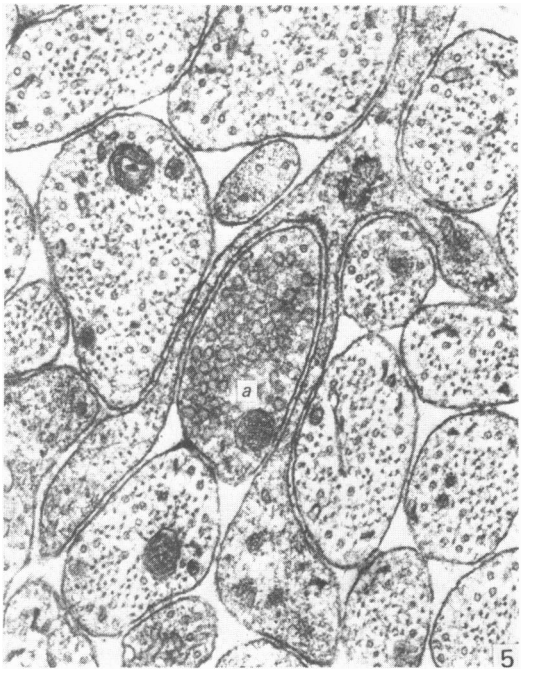

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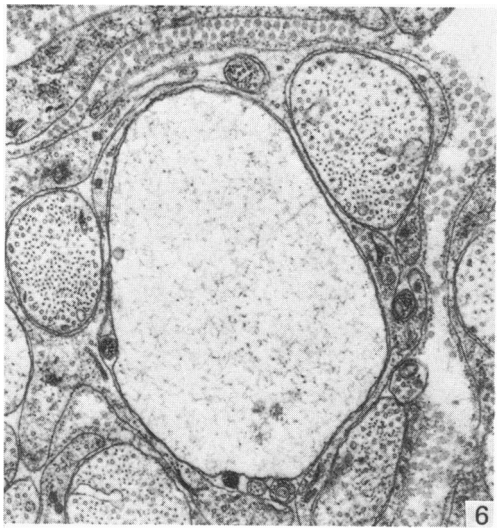


Fig. 6. 45 dpc. A swollen profile resembling a degenerating unmyelinated axon is ensheathed by a Schwann cell process together with some intact unmyelinated axons. × 24900.

First postnatal month

During the first three postnatal weeks, distorted and fragmented myelin sheaths were still found associated with 2–3 μm axons. In addition, irregular paranodal segments with myelin sheath outgrowths and myelin bodies, i.e. 'complex paranodes' (Berthold, 1968), were common around 3–4 μm axons during weeks 2 and 3. Four weeks after birth, such internodal and paranodal myelin irregularities were no longer seen. Cross sections through paranodes of 4–5 μm axons now showed a crenated shape with associated cords of Schwann cell cytoplasm rich in mitochondria. The nodal gap contained a corona of microvillus-like Schwann cell processes. These features, which are typical for the fully differentiated nodal–paranodal region of larger myelinated axons (Berthold, 1968), were not seen in smaller ones.

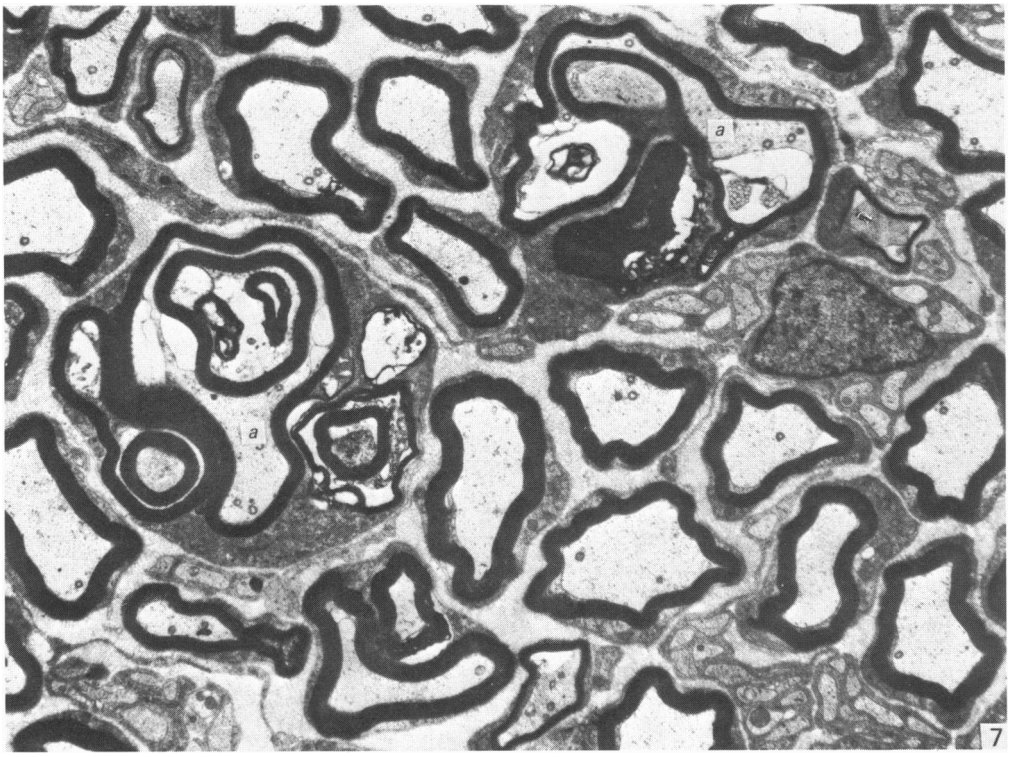
1 *month*–11 *years postnatally*

During the first weeks of the second postnatal month, *de novo* myelination continued. Six to eight weeks after birth, the inferior alveolar nerve seemed to be qualitatively mature, as judged from the lack of early myelinating axons and from the nodal–paranodal structure of larger and medium sized ($\geqslant$4–6 μm) myelinated axons. The growth in size, however, was far from completed. Specimens of the nerve

Fig. 7. Newborn. Among the myelinating axons two (*a*) display highly distorted myelin sheaths. Most of the unmyelinated axons occupy separate Schwann cell pockets. × 7200.

Fig. 8. Newborn. Longitudinal section. An axon with a highly distorted myelin sheath, corresponding to the labelled axons in Fig. 7, is seen. The short myelin sheath is intercalated between two nodes of Ranvier (*r*). *sn*, Schwann cell nucleus. × 3500.

Fig. 9. Newborn. Longitudinal section. A short, completely demyelinated axon segment is bordered by two seemingly intact paranodes (*pn*). Remnants of a degenerating cell (*) and lamellated bodies, most probably representing myelin debris, are seen along the demyelinated segment. × 6800.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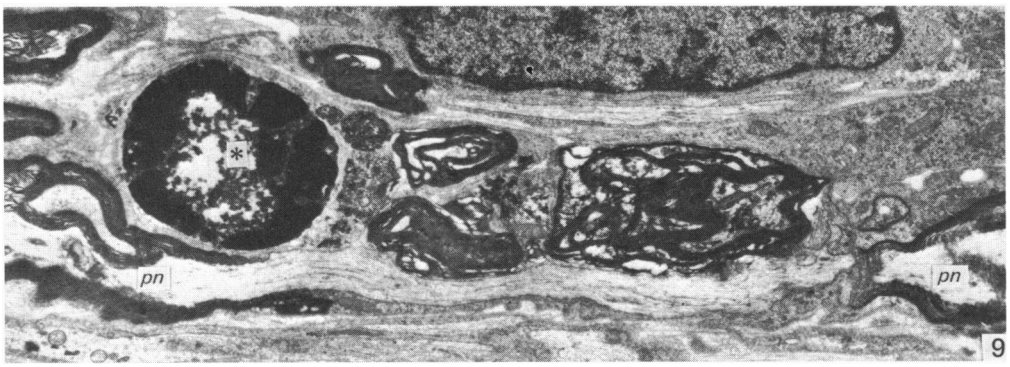
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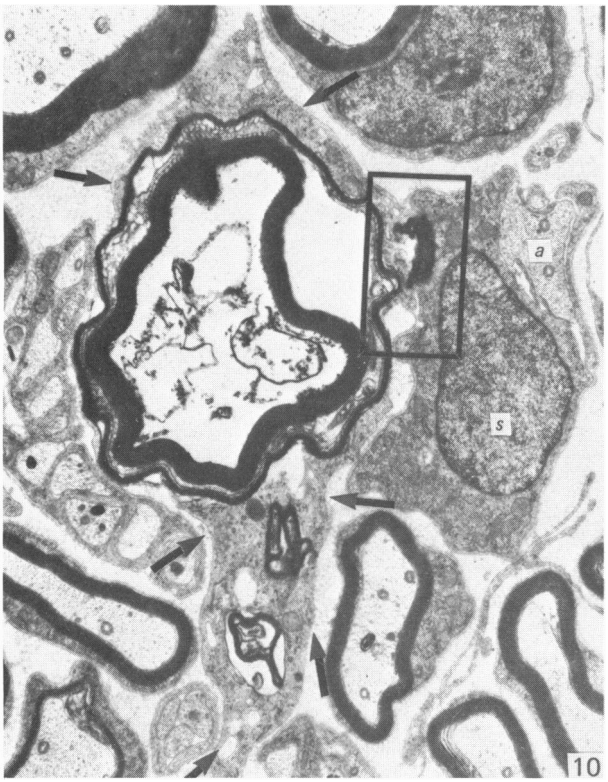


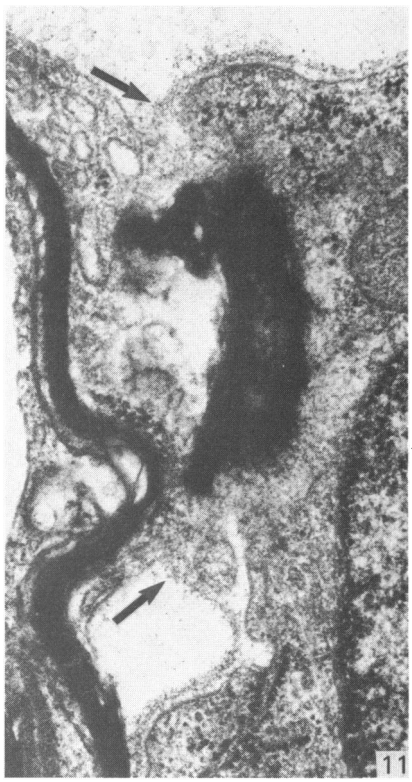


Fig. 10. Newborn. Arrows indicate process from a macrophage containing what seems to be myelin debris. A Schwann cell (*s*) ensheathing a fairly large non-myelinated axon profile (*a*) is closely associated with the process. A site of close contact (framed) is shown at higher magnification in Fig. 11. ×10700.

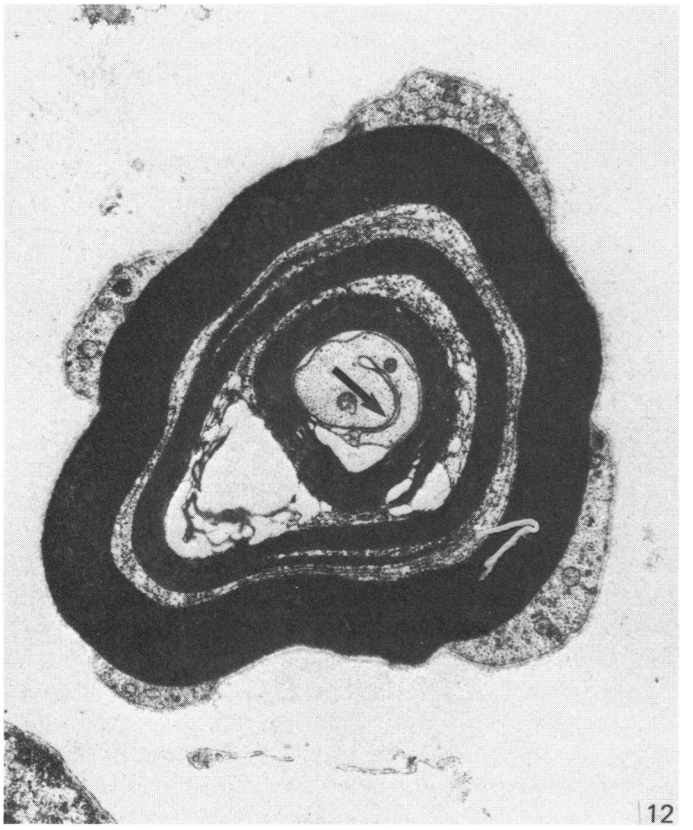
Fig. 11. Newborn. Detail of Fig. 10. Note that the continuity of the Schwann cell basement membrane appears to be interrupted at the site of intimate cellular contact (between arrows). ×52200.

from 3–11 months old animals showed no unusual features which could be related to the continuing shedding of primary teeth (Mohiuddin, 1950). In the adult animals aged 9 years or less, the nerve presented endo- and perineurial features in general agreement with previous descriptions of adult peripheral nerves (see, e.g., Bischoff & Thomas, 1975; Ochoa, 1975; Landon & Hall, 1976; Berthold, 1978). In the cats aged 10 and 11 years, however, axonal and perineurial alterations were found (Figs. 12–14). Some seemingly atrophic myelinated axons had disproportionately thick myelin sheaths (Fig. 12). Schwann cell extensions were sometimes invaginated into such axons (Fig. 12). Features indicating axonal regeneration and/or remyelination, as

Fig. 12. 11 years. A seemingly atrophic axon is surrounded by a thick myelin sheath subdivided into rings by cytoplasmic clefts. A Schwann cell expansion invaginates the axon (arrow). ×7800.

Fig. 13. 11 years. Schwann cell profile containing unmyelinated axons (*u*) and a collagen pocket (lower arrow). A Schwann cell profile lacking an axonal relation is indicated by the upper arrow. ×21800.

Fig. 14. 11 years. Perineurial cells with abnormally thick basement membranes. ×40900.



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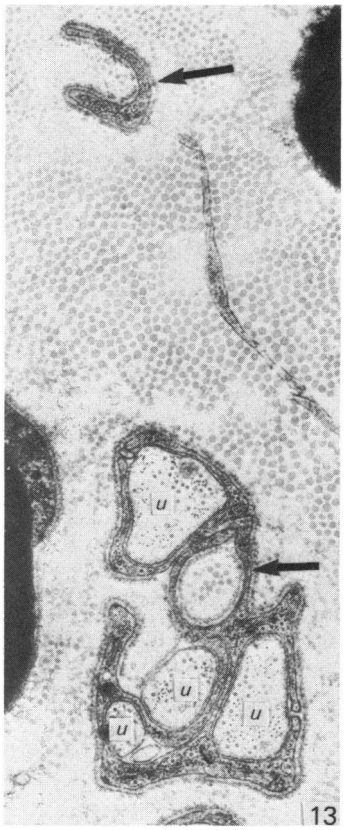

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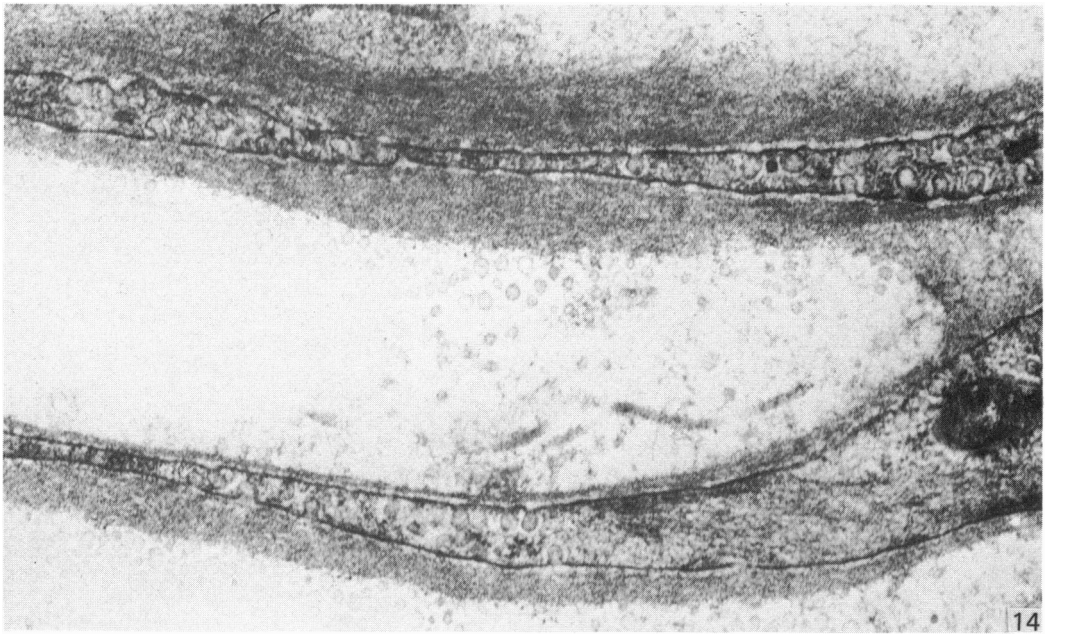

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well as continuing and past unmyelinated axon degeneration (Ochoa & Mair, 1969) were occasionally encountered (Fig. 13). In addition, the perineurial layers were covered by abnormally thick basement membranes (Fig. 14).

DISCUSSION

Most of the axons of the inferior alveolar nerve arise from parent neurons in the trigeminal ganglion, which appears 2 weeks after mating in the cat (Halley, 1955). A few days later, the mandibular division extends a short distance from the ganglion (Windle, 1932). As shown by our results, an inferior alveolar nerve composed of small axons and primitive sheath cells is present in the mandibular process at 25 dpc. In previous studies on fetal nerves, similar sheath cells have been regarded as immature Schwann cells (Gamble, 1966; Billings-Gagliardi, Webster & O'Connell, 1974). If these cells represent Schwann cell precursors, the area delimited by the circumferential layer would represent a labyrinthine periaxonal space common for all axons. Because no separate sheath existed outside the circumferential layer, as also described in fetal human ulnar nerves (Gamble, 1966), an endoneurium, in the true sense, had not yet appeared. That the circumferential cells at 25 dpc would represent immature perineurial cells seems unlikely (Gamble, 1966).

By 35 dpc, large groups of axons were collectively ensheathed by extensively branching cells with obvious Schwann cell features (Gamble, 1966; Ochoa, 1971). The presence of collagen fibrils in close relation to these cells and the lack of endoneurial fibroblasts support the view that Schwann cells may form collagen (see Mustafa & Gamble, 1978; Bunge *et al.* 1980). The transition from a collective ensheathment of the entire inferior alveolar nerve to ensheathment of axon bundles coincided with the appearance of flattened cells along the circumference of the nerve. This sheath was similar to the developing perineurium of the human fetal sural nerve (Ochoa, 1971), and defined an endoneurial space. The perineurial sheath showed an immature configuration throughout the prenatal period. Mature features, such as tight junctions (Gamble & Breathnach, 1965; Low, 1976), were first seen at birth. The tight junctions have been ascribed an important role in the perineurial barrier function (see Kristensson & Olsson, 1971). Their first appearance at birth might then be accompanied by a change in perineurial permeability.

The occurrence of what seemed to be degenerative alterations in several unmyelinated axons at 40–45 dpc suggests a developmental loss of axons in the inferior alveolar nerve, as described in some other nerves (see Aguayo, Terry & Bray, 1973; Sohal & Weidman, 1978; Mustafa & Gamble, 1979). However, on this point, quantitative studies are necessary to exclude the possibility of artefact. During the last 2 weeks before birth and the first 3 postnatal weeks, some of the larger myelinated axons showed short and distorted or completely disintegrated myelin sheaths. Similar observations in spinal roots of the cat and other mammals have been interpreted as a removal of some internodes of myelin from axons which are to become large (Berthold, 1968, 1973). This would allow the remaining internodes to grow more in length than the nerve trunk, which might influence conduction time (see Berthold, 1978). Our findings show that a corresponding process takes place in the developing inferior alveolar nerve. Features suggestive of a transfer of myelin fragments from Schwann cells to associated macrophages indicate that endoneurial macrophages may participate in myelin breakdown during developmental demyelination, as, for example, during Wallerian degeneration in the peripheral

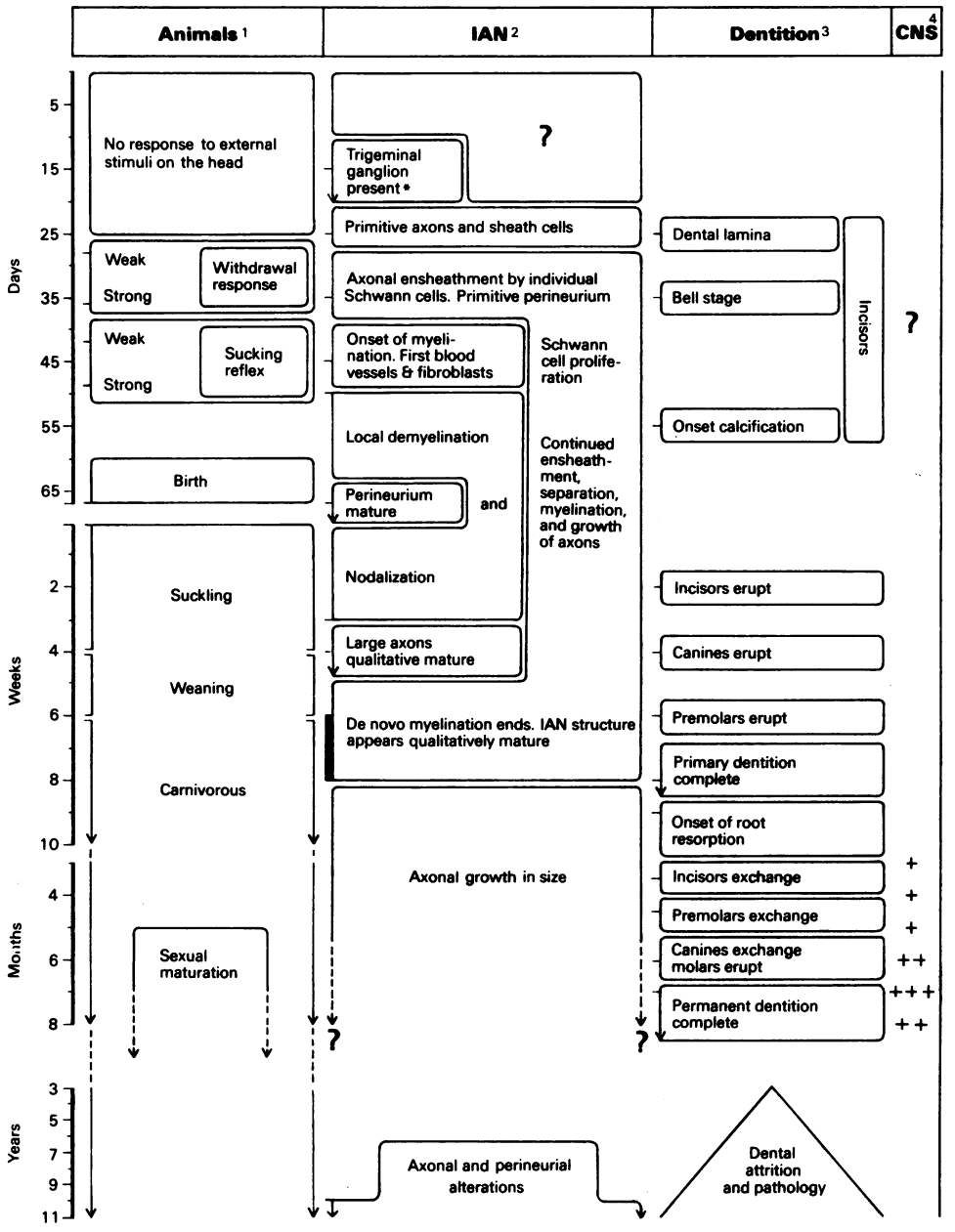

Fig. 15. Schematic description of the development of the inferior alveolar nerve (IAN) correlated with the behavioural and dental development. [1] Windle & Griffin (1931), Stein (1975), Hamilton & Carroll (1977). [2] Present observations and (*) Halley (1955). [3] Shapiro (1930) and own observations. [4] Terminal degeneration in brain stem (Westrum & Canfield, 1979).

nervous system (see Allt, 1976). Four weeks after birth, the nodal–paranodal region of large axons of the inferior alveolar nerve ($\geqslant 5$ μm) appeared qualitatively mature, as defined by Berthold (1968, 1978). In developing feline spinal nerves, demyelination and nodalization is accompanied by a functional maturation. It was proposed that the attainment of mature conduction properties is somehow related to the qualitative structural maturation (see Skoglund, 1969; Berthold, 1978). If a similar relation is valid for the inferior alveolar nerve, then the larger myelinated axons would be functionally mature about 4 weeks after birth. With respect to its smaller axons, separation and *de novo* myelination continued until 6–8 weeks postnatally. At this time, the qualitative maturation of both large and small axons appeared to be complete, although increase of axonal diameter proceeds for several months after birth (Mohiuddin, 1951).

The shift from the primary to the permanent dentition takes place between months 3 and 7 (Shapiro, 1930). Before shedding, the pulpal axons of primary teeth degenerate (Bradlaw, 1936; Mohiuddin, 1950). Changes interpreted as terminal degeneration have also been observed in the brain stem during and some time after shedding of primary teeth (Westrum & Canfield, 1979). During this period, the axons of the inferior alveolar nerve increased slowly in size and no unusual features were seen. Consequently, axonal degeneration related to tooth shedding seems to be limited to the terminal branches (cf. Mohiuddin, 1950).

In the oldest animals, some altered myelinated axons and occasional signs of axonal degeneration and loss were found. A loss of neuronal perikarya and peripheral axons in senescence has been described in various species (see Ochoa & Mair, 1969; Samorajski, 1974). It cannot be excluded that some axonal degeneration in the old inferior alveolar nerve is secondary to neuron death. However, axonal atrophy and adaxonal Schwann cell protrusions typically occur proximal to neurotomies and in amputation neuromata (Aitken & Thomas, 1962; Morris, Hudson & Weddell, 1972; cf. Spencer & Thomas, 1974). The presence of similar changes in the ageing inferior alveolar nerve might then be secondary to pathological alterations in the dentition, partial tooth loss and pulpal involution, which is seen in old cats (Fried & Hildebrand, unpublished observations). In addition, the old inferior alveolar nerve showed unusually thick perineurial basement membranes (cf. Gamble & Eames, 1964). Pathological thickening of basement membranes of peripheral nerves has been described in relation to Schwann cells and endothelial cells in diabetic neuropathy (Thomas & Eliasson, 1975), and seems to occur along perineurial cells in Fabry's disease (Fig. 67 in Bischoff, 1970).

In Figure 15, the maturation of the inferior alveolar nerve, discussed above, has been summarized and correlated to the behavioural and dental maturation. The inferior alveolar nerve first appears to attain functional significance about four weeks after conception, when tactile stimuli anywhere on the head elicit a withdrawal response (Windle & Griffin, 1931). At that time, there is a shift from a collective ensheathment of all axons of the nerve by primitive sheath cells to ensheathment of axonal compartments by individual immature Schwann cells. Establishment of the sucking reflex (Windle & Griffin, 1931) coincides in time with initial myelination in the nerve around 45 dpc. At birth, the kitten starts sucking, which demands an efficient afferent link from the oral region. In the inferior alveolar nerve, myelination has been in progress for at least 3 weeks, local demyelination of larger axons is proceeding and small axons undergo primary ensheathment. During the first month, the primary dentition partly erupts (Shapiro, 1930) and the kitten enters the weanling

period (Hamilton & Carroll, 1977). Simultaneously, *de novo* myelination of small axons continues, demyelination ceases, and nodalization of larger axons is completed. Eight weeks after birth, the primary dentition becomes fully developed (Shapiro, 1930), the inferior alveolar nerve is qualitatively mature and the weanling period has just ended (Hamilton & Carroll, 1977). The shift from the primary to the permanent dentition 3–7 months postnatally does not elicit any changes in the inferior alveolar nerve. The deterioration of the permanent dentition in old age is accompanied by axonal and perineurial changes.

SUMMARY

The qualitative structural development of the inferior alveolar nerve was studied by electron microscopy in 56 pre- and postnatal kittens and 21 young and old adult cats. At 25 days post conception the nerve was composed of a bundle of small axons enclosed by primitive sheath cells. Three weeks later myelination had been initiated. Axons measuring 2–3 μm underwent local demyelination from 2 weeks before to 3 weeks after birth. This was accompanied and followed by nodalization of larger axons. A typical perineurium was first apparent in the newborn kitten. Six to eight weeks postnatally, the nerve appeared qualitatively mature, although axonal growth was far from completed. This coincides with achievement of a fully mature primary dentition shortly after the weanling period. Apart from a continued size growth, no changes were observed in the nerve during the transition from the primary to the permanent dentition. In the inferior alveolar nerve of old cats, axonal and perineurial changes co-existed with signs of dental attrition and pathology.

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