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Intranuclear inclusions in Schwann cells of aged fowl ciliary ganglia

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INTRODUCTION

The occurrence of intranuclear inclusions in the form of filamentous structures, bands, rodlets, or paracrystalline aggregates in both plant (Wergin, Gruber & Newcomb, 1970) and animal cells (de Thé, Rivière & Bernhard, 1960) has long been known to microscopists. In nervous tissue rod-shaped intranuclear bodies were first reported by the end of the last century (Mann, 1895; Roncoroni, 1895). The inclusions were described as elongated, straight or crescentic bodies and were thought to be a special feature of the nerve cells only.

Since the study of Siegesmund, Dutta & Fox (1964), who first described the ultrastructure of intranuclear fibrillar aggregates found in certain neurons of various brain regions in the squirrel monkey and the rabbit, several papers on the electron microscopic features of intranuclear inclusions in nerve cells have been published. These inclusions are easily distinguishable from the nucleolus and chromatin. They occur in two main forms: orientated filaments, in certain cases associated with microtubules or arranged so regularly as to form a lattice (Seïte *et al.* 1979; Brion, Couck & Flament-Durand, 1982; Bianchi & Gioia, 1985), and small ovoidal bodies composed of a microfilamentous capsule containing a central granular component (Bouteille, Kalifat & Delarue, 1967; Schochet, 1972).

It has been demonstrated that the frequency of nuclear inclusions varies from region to region of the nervous system and from one animal species to another, and may be age-dependent (Dahl, 1970; Field & Peat, 1971; Feldman & Peters, 1972; Johnson & Miquel, 1974; David & Nathaniel, 1978; Vuillet-Luciani, Vio, Cataldo & Seïte, 1979; Fotheringham & Davies, 1980; Toper *et al.* 1980; Brion *et al.* 1982; Lafarga, Crespo & Villegas, 1983; Curcio, McNelly & Hinds, 1984; Fernandez, Suarez & Gutierrez, 1984). A survey of the literature shows that the inclusions seem to be far more frequent in post-mitotic nerve cells than in any other non-neuronal cell type; additionally, the inclusions appear particularly rare in both central and peripheral myelin-forming cells (Dahl, 1970).

During an extensive investigation on age-dependent ultrastructural changes occurring in the avian peripheral ganglia (Fiori & Mugnaini, 1979; Mugnaini & Fiori, 1987), an increase in the occurrence of intranuclear inclusions, sometimes associated with the so-called 'pseudo-inclusions', was noted in Schwann cells of older fowls. This paper describes such inclusions and some additional age-related ultrastructural alterations in the parasympathetic ciliary ganglion.

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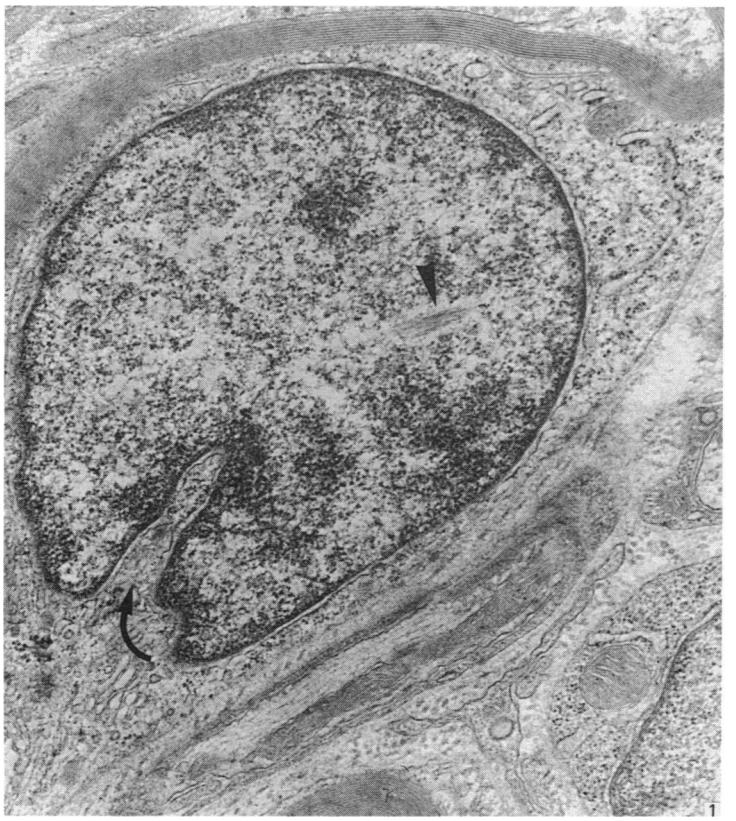

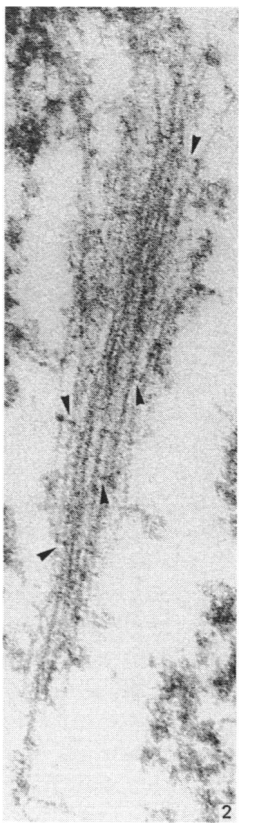

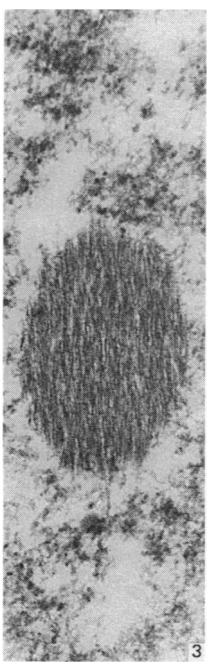

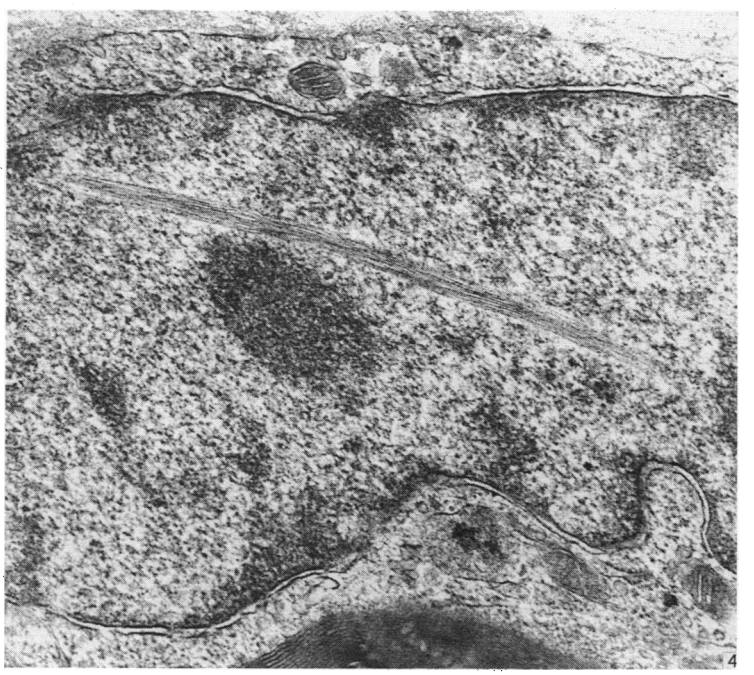

MATERIALS AND METHODS

Electron microscopic observations were carried out on 21 chickens (*Gallus domesticus* L.) aged between one day post-hatching and seven years. Animals were provided by SPAFAS, Inc., Norwich, CT, by the Department of Pathobiology, University of Connecticut, Storrs, CT, and by local farms; they were all healthy and without obvious signs of diseases at the time of killing.

The birds were anaesthetised either with halothane vapour or by injecting 0·1–0·3 mg/kg^{-1} of 6·5% sodium pentobarbitone into the subalar vein. They were then perfused via the ascending aorta with a buffered saline solution followed without interruption by a fixative containing 1–3% paraformaldehyde and 1·25–3% glutaraldehyde in 0·12 M phosphate buffer, pH 7·4, at 37 °C. In some cases this fixative was followed, always without interruption, by a second one containing 2·5% glutaraldehyde in 0·12 M phosphate buffer, pH 7·5, at room temperature. About one hour after the end of perfusion, both right and left ciliary ganglia were dissected out under an operating microscope, postfixed in 2% osmium tetroxide in phosphate buffer, stained *en bloc* with uranyl acetate and embedded in a TAAB resin-Epon mixture (Cantino & Mugnaini, 1975). Ultrathin sections were stained with uranyl acetate and lead citrate and photographed in a Zeiss EM10 electron microscope operated at 80 kV.

RESULTS

The cytological features of the avian ciliary ganglion at both light and electron microscopic levels have already been extensively described (Terzuolo, 1951–1952; Landmesser & Pilar, 1970; Marwitt, Pilar & Weakly, 1971; Cantino & Mugnaini, 1974, 1975; Mugnaini & Fiori, 1987) and will not be repeated here. As in other autonomic and spinal ganglia, in the ciliary ganglion Schwann cells are easily distinguishable, on the basis of their association with myelin sheaths and basal laminae, from connective tissue elements and other peripheral glial cells, such as perineuronal satellite cells and small, electron-dense cells identified as microglial elements (Fiori & Mugnaini, 1981).

Intranuclear rod-like and ovoidal inclusions were observed in many Schwann cells of the oldest fowls (five 5 years old and two 7 years old animals) and were absent from neurons and other cell types, except for rare inclusions which occurred in satellite cells of ciliary neurons. Ultrathin sections taken at random through different levels of ciliary ganglia in chickens aged less than one year failed to show similar intranuclear bodies

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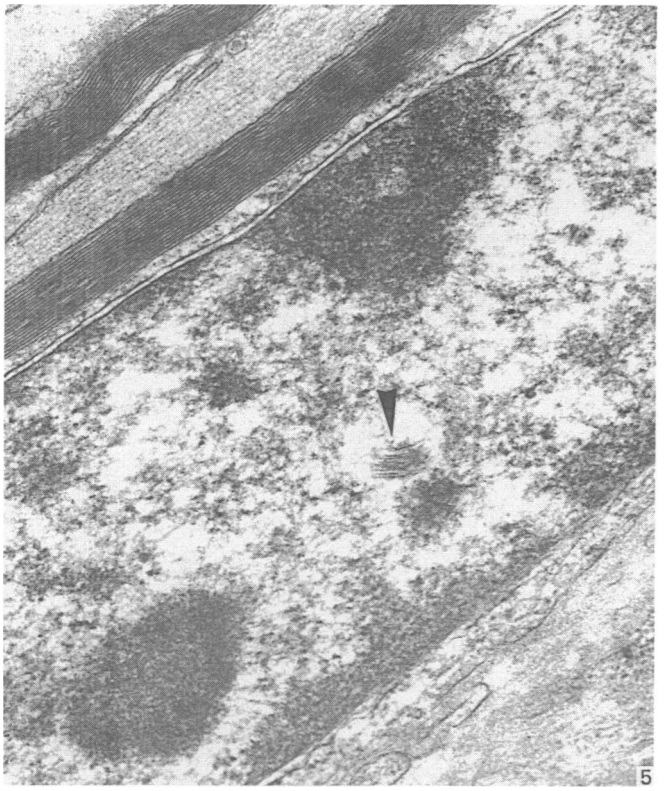
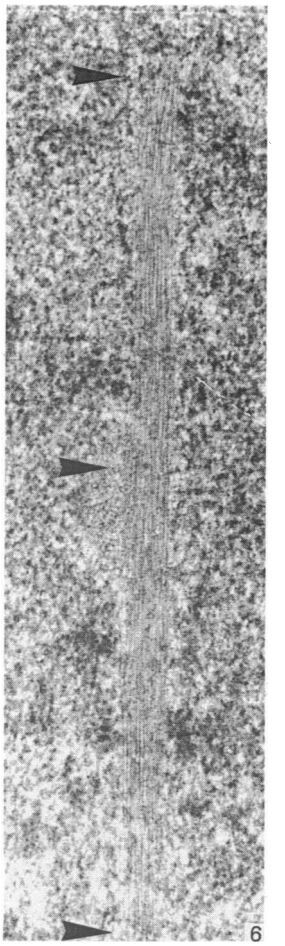
Fig. 1. Schwann cell from a 5 years old fowl. The curved arrow points to a fairly deep indentation of the nucleus which still retains an overall ovoidal shape. A rodlet composed of about a dozen filaments and surrounded by a halo of electron-transparent nucleoplasmic matrix is indicated by the arrowhead. × 18200.

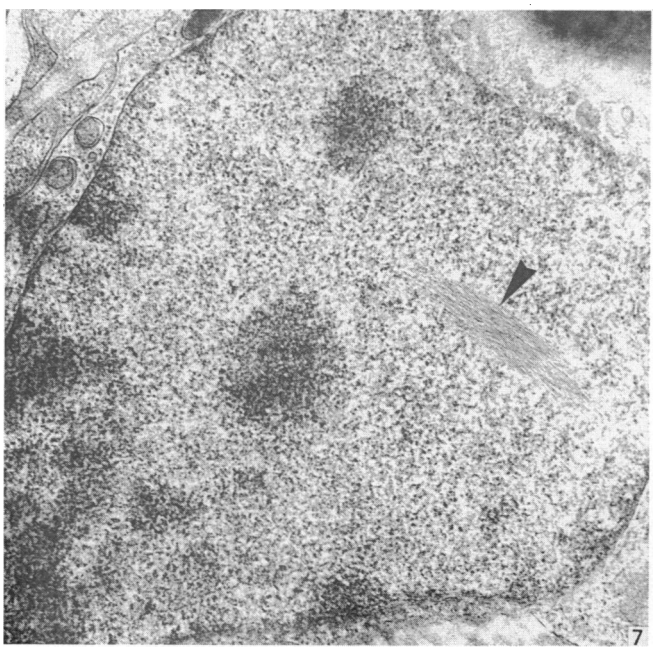
Fig. 2. High magnification of a nuclear rodlet from a different Schwann cell of a 5 years old fowl. Filaments are unevenly distributed within the bundle and display some spiny lateral branches (arrowheads). × 50750.

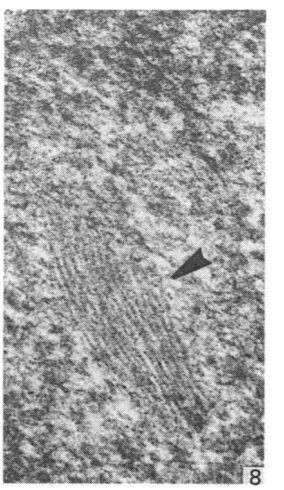
Fig. 3. A rather compact bundle of nuclear filaments cut along an oblique plane in a Schwann cell nucleus from a 7 years old fowl. × 47600.

Fig. 4. Schwann cell from a 7 years old fowl. A long, slightly arched filamentous bundle runs close to the nucleolus. Except in a few places an electron-transparent halo separates the rodlet from other nuclear components. The nucleus tends to have an irregular profile although deep invaginations are absent. × 19320.









in either neuronal or non-neuronal cells, including Schwann cells. Only occasional inclusions were found in Schwann cells of chickens 1–4 years old.

Short series of sections (5–10) were therefore taken only from ciliary ganglia of the oldest fowls to elucidate features of the inclusions and the possible occurrence of more than one inclusion per nucleus. In single sections, the ratio of the number of inclusions and the number of Schwann cell nuclei was found to be approximately 1:4. Often, apparently negative nuclei were seen to contain inclusions when serial sections of the same cells were analysed. It was concluded, therefore, that inclusions probably occur in the majority of Schwann cells.

The intranuclear inclusions appeared to be of two types: spindle-shaped bundles of filaments and ovoidal granulofibrillary bodies (Figs. 1–16). According to the nomenclature of nuclear bodies suggested by Bouteille *et al.* (1967) and subsequently modified by Ghadially (1975) and Seĭte *et al.* (1979), only the first type could be classified as an intranuclear inclusion proper, the second being considered an associated structure. However, granulofibrillary bodies have been described as a separate entity by several authors (Grunnet, 1975; Schochet, 1972; Fernandez *et al.* 1984) and occur in a variety of forms. It is proposed that granulofibrillary bodies too should be considered as 'intranuclear inclusions' because they sometimes occur in the absence of the filamentous bundles (see below). The filament bundles and the granulofibrillary bodies will be called henceforth Type I and Type II respectively, without any particular reference to previous classifications.

In several cases the two types of inclusion occurred in the same nucleus (Figs. 13–16) and sometimes were spatially associated. Most often, however, only one inclusion was observed in individual nuclei. In a few cases a Type II inclusion surrounded by microtubule-like structures was seen (Figs. 12, 16). Both types of inclusions usually appeared to be separated from other nuclear components, such as chromatin, the nucleolus, or the nuclear envelope, by an electron-transparent halo nearly free from chromatin granules.

The bundles of filaments were usually straight, sometimes slightly arched, and tapered at both ends, reaching a maximal length of about 5·2 μm (Fig. 4), and ranging between 120 and 320 nm in width. The filaments, never arranged in a paracrystalline structure, always seemed shorter than the inclusion itself (Fig. 2), but their true length was difficult to ascertain in thin sections (Seĭte *et al.* 1979). Within the bundles the filaments were arranged parallel to one another, and were orientated in the long axis of the bundle (Figs. 1–8). Individual filaments were 6–8 nm thick and were spaced by 4–12 nm-wide intervals. The overall number of filaments constituting an individual bundle appeared to vary considerably, ranging from about 10 to more than 40 (Fig. 3). In some cases bridge-like structures were observed to cross the narrow cleft between two individual filaments (Fig. 2); this feature was reminiscent of previous reports by other authors showing the presence of lateral spines or side-arms inter-

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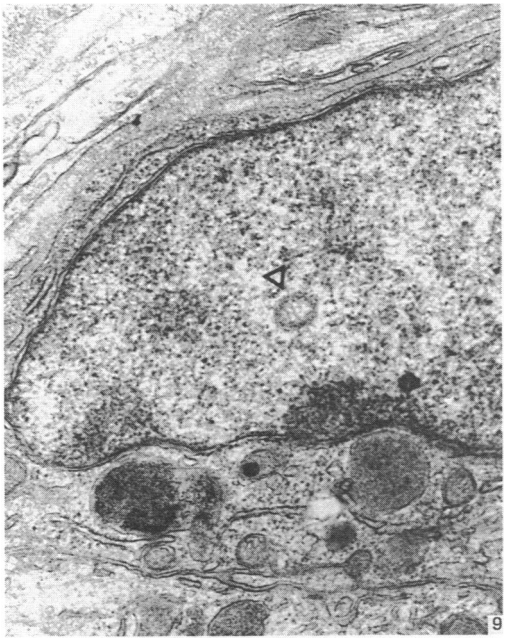
Fig. 5. Schwann cell from a 5 years old fowl. The filamentous bundle (arrowhead) is orientated along a main axis different from that of the cell (and of the related myelinated axon in the upper left corner). × 21000.

Fig. 6. Nucleus of a Schwann cell from a 5 years old fowl with a longitudinally cut rodlet (arrowheads). Several chromatin granules are packed in close proximity to the inclusion. × 15000.

Fig. 7. This rodlet (arrowhead) from another Schwann cell of a 5 years old fowl appears to be 'embedded' in a nuclear area densely provided with chromatin granules. Such an inclusion may be barely distinguishable when scanned at low magnification. Single filaments show a regular periodicity of about 2 nm. × 12125.

Fig. 8. Another example of intranuclear rodlet (from a 5 years old fowl) completely surrounded by chromatin granules. × 52500.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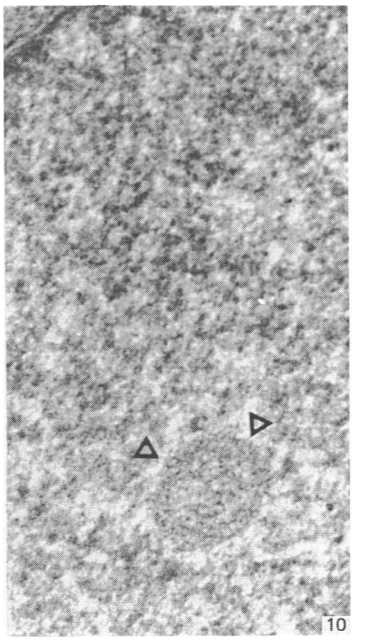
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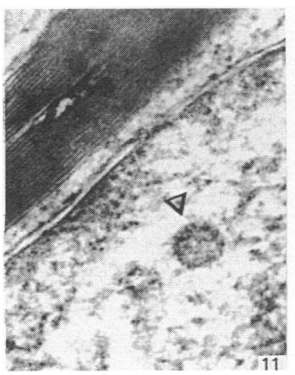
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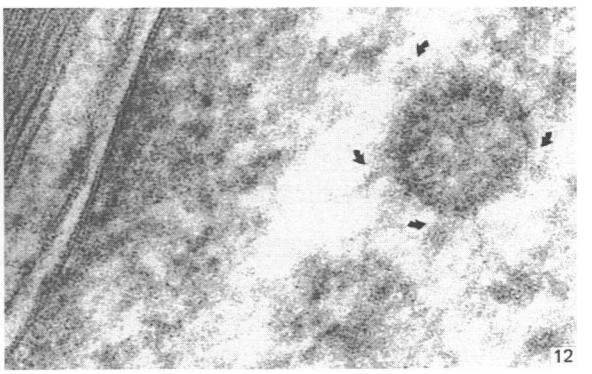
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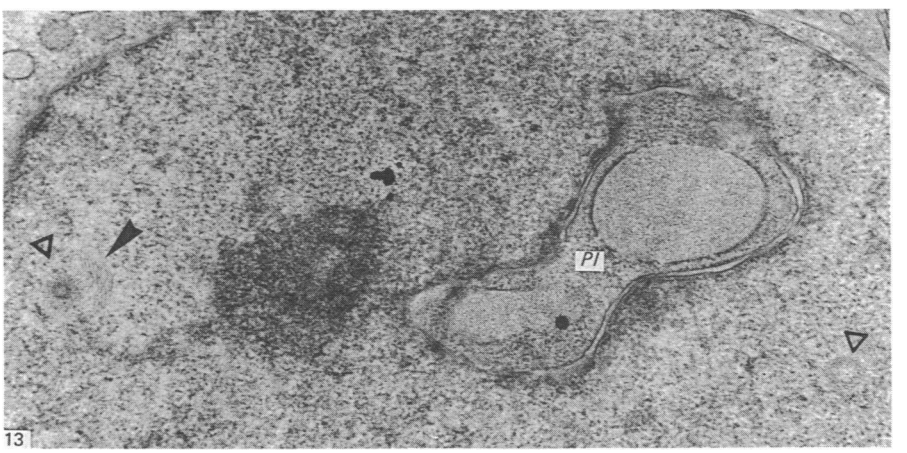

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connecting the filaments in a number of bundles. As described in other papers (Mugnaini, 1967; Masurovsky, Benitez, Kim & Murray, 1970; Peters, Palay & Webster, 1976), close proximity between filamentous rodlets and dense granular bodies was occasionally observed. In the sections cut along a plane coincident with the main axis of the bundle, a 2 nm periodicity was seen in the individual filaments (Figs. 6–8), suggesting the presence of an internal substructure.

Type II inclusions appeared to be composed of a cortex of one or two layers of very thin filaments surrounding a centre sometimes clearly occupied by either a microfilamentous network or fine and coarse granules, or apparently containing only a cottony matrix (Figs. 9–12). The diameter of these inclusions was approximately 350 nm. In some instances, their electron density was so low as to make their recognition rather difficult (Fig. 10). As in the case of Type I inclusions, those of Type II were usually separated from the other nuclear components by a halo, although chromatin granules could make contact with the inclusions at points.

With the increasing age of the animal, the nuclear shape, which in the Schwann cells is usually ovoid, tended to become irregular by the occurrence of indentations or deep invaginations (Figs. 1, 4, 9, 15). According to the plane of the section, cytoplasmic extensions into the nucleus were sometimes seen as pseudo-inclusions which appeared occasionally in association with both types of 'true' inclusions (Fig. 13). The pseudo-inclusions contained microtubules, polyribosomes, and dilated cisterns of the rough endoplasmic reticulum.

Besides the occurrence of intranuclear inclusions and pseudo-inclusions, few abnormal features were found in the cytoplasm of Schwann cells of the oldest fowls. Occasionally, mitochondria with angular or wavy cristae were encountered in the cell body (Fig. 19). In the inner turn of the myelin sheath, which contains Schwann cell cytoplasm, filamentous masses were observed in random sections. These filamentous assemblies, covered by the Schwann cell plasma membrane, protruded into the adaxonal space and indented the contour of the axon (Figs. 17, 18).

DISCUSSION

Schwann cells of parasympathetic ciliary ganglia of aged fowls were found to contain numerous intranuclear inclusions. These were morphologically identical to those already described in various types of neurons and glial cells (for a review of the literature, see Payne & Nagle, 1983). Similar inclusions have also been described in a wide variety of non-neural cells under normal and pathological conditions (Wyatt, Schochet & McCormick, 1970; Graham, Payne & Nagle, 1981; Ishizeki, Tachibana, Sakakura & Nawa, 1981; Yasuzumi *et al.* 1981, 1982; Thiele & Mahrle, 1983).

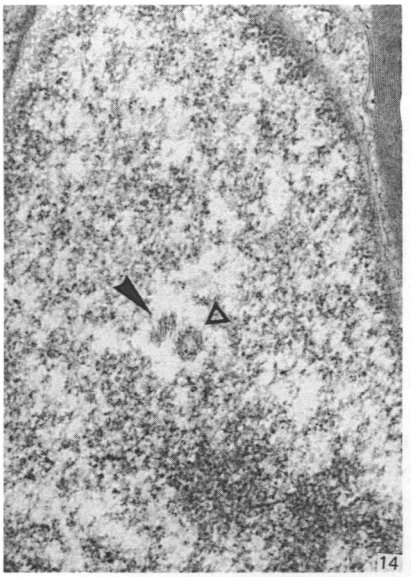
The frequent occurrence of two or more inclusions of different structure in the same

Fig. 9. Schwann cell from a 7 years old fowl. A granulofilamentous body is seen in the nucleus (triangle). The cytoplasm contains some single membrane-bound dense bodies, probably lysosomes, the position of which modifies the otherwise regular nuclear contour. × 14700.

Fig. 10. This granulofibrillar body (from a Schwann cell of a 5 years old fowl) appears to be completely surrounded by chromatin granules (triangles). × 49000.

Figs. 11–12. A granulofibrillar body (triangle) seen at two different magnifications (Fig. 11, × 21700; Fig. 12, × 43750), from a Schwann cell of a 5 years old fowl. The inclusion contains a cottony granular matrix and displays radial appendages (curved arrows) which seem fairly evenly spaced from each other.

Fig. 13. Schwann cell from a 7 years old fowl. A pseudo-inclusion (*PI*) containing distended vesicles of the rough endoplasmic reticulum and a granular matrix is shown together with two granulofibrillar bodies (triangles) and an obliquely-cut rodlet (arrowhead). The two granular bodies differ in their fine structure. × 17500.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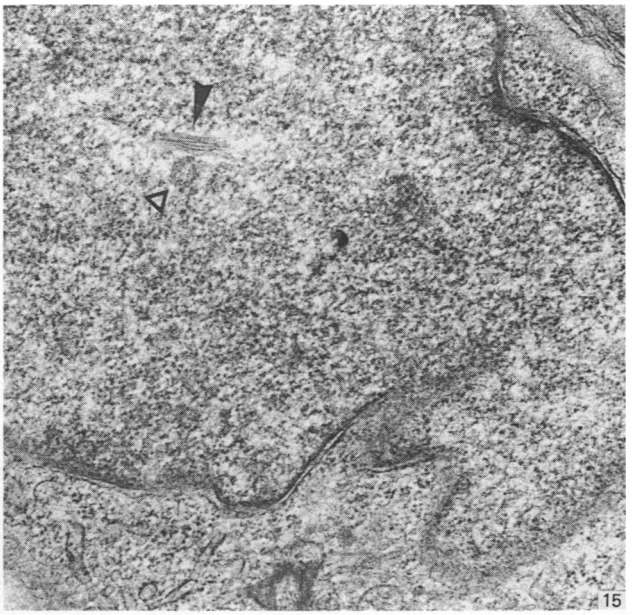
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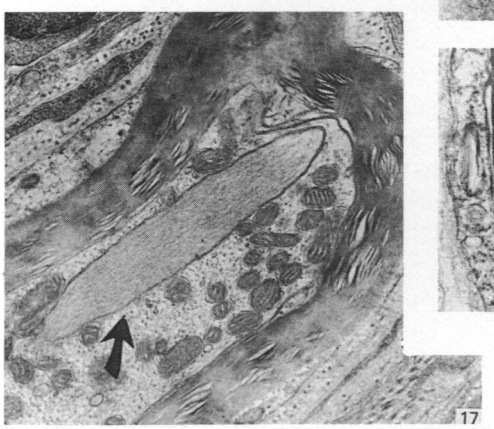
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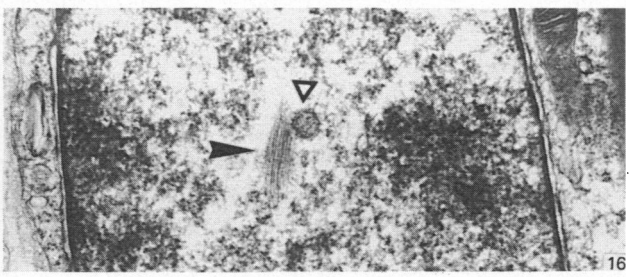
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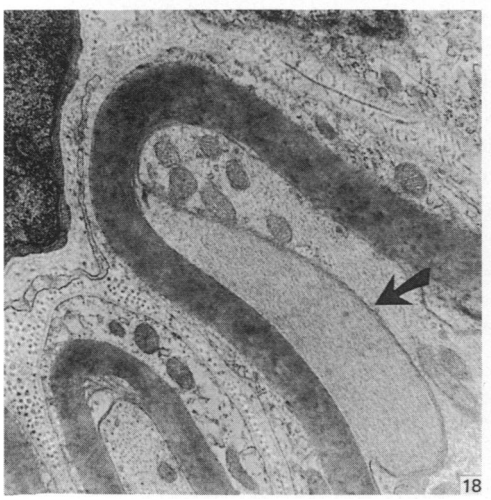
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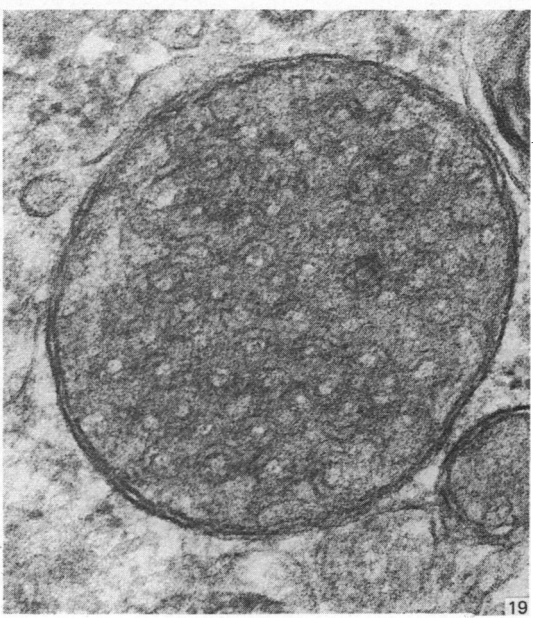
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nucleus and the simultaneous presence of inclusions and pseudo-inclusions are noteworthy features, stressed in few other studies (Mugnaini, 1967; Uehara & Ueshima, 1984). The most striking aspects of the observations reported in the present paper, however, remain the high frequency of occurrence of nuclear inclusions in Schwann cells of old fowls and the nearly complete restriction of the inclusions to this cell type in the ciliary ganglion. Intranuclear inclusions in myelin-forming cells have not been reported previously, except for the isolated observation of Dahl (1970).

Seïte and his collaborators (Zerbib, Vuillet-Luciani, Escaig & Seïte, 1973; Seïte *et al.* 1979; Vuillet-Luciani *et al.* 1979), who studied the sympathetic stellate, superior cervical and coeliac ganglia of the cat, found intranuclear rodlets in the neurons but not in non-neuronal cell types. Also Costa & Paula-Barbosa (1979), in an ultrastructural study of the parasympathetic sphenopalatine ganglion of the dog, observed typical rodlets only in the neurons.

In the present study, the occurrence of intranuclear inclusions appears to be clearly an age-dependent phenomenon. This feature is demonstrated by the increased frequency of intranuclear rodlets and granulofibrillar bodies in fowls aged five to seven years. An increased frequency of intranuclear inclusions had been reported in different types of nerve cells by Field & Peat (1971), Feldman & Peters (1972), Vuillet-Luciani *et al.* (1979), Fotheringham & Davies (1980), Brion *et al.* (1982), Curcio *et al.* (1984) and Fernandez *et al.* (1984). All these studies have stressed that intranuclear inclusions are exceedingly rare in young animals, but relatively frequent in adult or old animals. Interestingly, rod-like inclusions have also been observed in senile dementia of Alzheimer type (Grunnet, 1975; Toper *et al.* 1980) as well as in infectious diseases, such as subacute sclerosing panencephalitis (Périer, Vanderhaeghen & Pelc, 1967), Creutzfeldt–Jakob disease (Grunnet, 1975) and scrapie (Chandler & Willis, 1966), which have been claimed to be somehow similar to Alzheimer's disease. On the other hand, intranuclear inclusions of various morphology have been described in conditions as diverse as oculopharyngeal muscular dystrophy (Martin, Ceuterick & Mercelis, 1982; Coquet *et al.* 1983), multiple myeloma (Vital *et al.* 1982), diabetic polyneuritis (Vallat *et al.* 1983), and liver carcinoma (Gibson, 1982), in which no age dependence may be demonstrated.

Unfortunately, no extensive study has so far been carried out on the features of ageing Schwann cells, since the investigators have paid attention mainly to the changes occurring in the central nervous system or to the modifications in axons and myelin sheaths of peripheral nerves. Likewise, the papers dealing with ageing in animal and human ganglia have not focused on possible nuclear changes taking place in Schwann cells (Ohta, Offord & Dick 1974). Therefore it remains undetermined whether the

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Fig. 14. Another Schwann cell from a 7 years old fowl. A typical rodlet (arrowhead) and a granulofibrillar body (triangle) are simultaneously present. Both of them are separated from other nuclear structures by a wide electron-transparent halo. × 17500.

Fig. 15. In this nucleus from a 5 years old fowl the two inclusion types (arrowhead and triangle) are surrounded more closely by chromatin granules. The nucleus is beginning to appear lobulated. × 19600.

Fig. 16. Paired inclusions in a Schwann cell from a 7 years old fowl. The granulofibrillar body (triangle) associated with a rodlet (arrowhead) is provided with several radial appendages. × 19075.

Figs. 17–18. Fibrillary accumulations (arrows) in the adaxonal cytoplasm of Schwann cells from a 5 (Fig. 17) and a 7 years old fowl (Fig. 18). Except for mitochondrial clustering, the axoplasm appear normal and no myelin changes are detectable. × 12600.

Fig. 19. Mitochondrion with curly cristae in a Schwann cell (from a 7 years old fowl) devoid of intranuclear inclusions. × 55300.

present findings in the ciliary ganglion are an age-dependent phenomenon confined to birds or whether they are present elsewhere in different animal species. Age-related alterations of Schwann cells could eventually have an adverse effect on the maintenance of axons, in view of the recently demonstrated glial–axonal interrelationships as well as the exchanges taking place between Schwann cells and the extracellular matrix (Bunge & Bunge, 1983).

A considerable number of Schwann cells in the ganglia examined in the present study showed pseudo-inclusions together with filamentous or granulofibrillar inclusions. Pseudo-inclusions have in the past been described as structures commonly associated with neoplastic processes (Flaks & Flaks, 1970; Glant, Berger & Davey, 1984) and, according to Leduc & Wilson (1959), represent an attempt by enlarged and/or fast-reproducing cells to maintain the normal ratio of nuclear surface area to nuclear volume; actually, in cancer cells the nuclear volume tends to increase following the overall demands for enhanced protein synthesis. This view is partly supported by the frequently observed presence of engulfed cisterns of rough endoplasmic reticulum as the most impressive organelle in the pseudo-inclusions, and by the close structural association of pseudo-inclusions with the nucleolus (Burns, Soloff, Hanna & Buxton, 1971; Bourgeois, *et al.* 1979). According to other authors, however, the pseudo-inclusions of certain cell types and tumours are the expression of non-specific nuclear irregularities, such as angulation and lobulation (DeLellis, Suchow & Wolfe, 1980). Since the Schwann cells in the present material, on the contrary, had only slightly abnormal nuclear shapes, it is possible that nuclear inclusions and pseudo-inclusions represent coincident epiphenomena depending on age-related variations of both intranuclear molecules and nuclear envelope, as reported in the neurons (Nosal, 1979; LaVelle & Buschmann, 1983). In this connection, it should be noted that not only inclusions, but pseudo-inclusions as well seem to increase numerically during life (Hirabayashi, Shimokawa, Ikeda & Orthner, 1979).

Recent reports emphasise that a large number of different experimental situations may induce the appearance of intranuclear bodies or a manifold increase in their frequency (Seĭte *et al.* 1971, 1973, 1977). The pharmacological and physical manipulations which have proven to be most effective in giving rise to intranuclear bodies included chronic alcohol administration (Tavares & Paula-Barbosa, 1981; Volk & Maletz, 1985), oestrogenic stimulation (Fitzgerald & Padykula, 1983; Padykula & Pockwinse, 1983), mitogen administration (Chaly, Setterfield, Kaplan & Brown, 1983) and heat-shock treatment (Welch & Suhan, 1985). It is noteworthy that these experimental conditions may either result in high rates of cellular protein biosynthesis or may impair protein metabolism at different (post-)translational levels. When specific inhibitors of protein synthesis, such as puromycin (Gambetti & Gonatas, 1967) and cycloheximide (Seĭte, Mei & Vuillet-Luciani, 1973) are administered, the presence of intranuclear inclusions is not affected, suggesting that they are relatively stable and probably formed from pre-existing protein subunits. Recent immunocytochemical studies using monoclonal antibodies raised against the nuclear matrix, nucleolar complex, nuclear bodies and filamentous elements in axons and some perikaryal regions of the adult avian dorsal root ganglia (Masurovsky & Fields, 1984) have confirmed that at least the granulofibrillar nuclear inclusions (Type II of the present study) possess epitopes with immunostaining properties similar to those of fibrillary components present in the neuronal cytoplasm and/or processes; this finding should rule out previous hypotheses about a DNA/RNA nature of the inclusions (Yasuzumi *et al.* 1981; Payne & Nagle, 1983; Topilko, Zakrzewski, Pichard & Viron, 1984; Penner & Tritt, 1985).

In any case, the simultaneous presence of 'true' inclusions and pseudo-inclusions in the same cell strongly supports the hypothesis that both of them are the expression of nuclear or cellular hyperactivity. Since the ciliary ganglia of aged fowls show a remarkable rearrangement in the extracellular components of the connective tissue (Fiori & Mugnaini, unpublished observations), Schwann cells could contribute to this changing pattern through the production of collagen or basal lamina materials (Bunge & Bunge, 1983); however, as is shown by the occurrence of filamentous masses in the Schwann cell cytoplasm adjacent to the axon (Figs. 17, 18), an enhanced level of cellular activity might also be explained by assuming that the Schwann cells in the ageing ciliary ganglion modify either the myelin turnover or their interactions with the axons. It would, therefore, be interesting to carry out further studies in experimental conditions and to extend the investigations to ciliary ganglia and nerves of other animal species at different ages.

SUMMARY

Schwann cells in ciliary ganglia of fowls aged five to seven years were found to contain numerous intranuclear inclusions and pseudo-inclusions. Similar inclusions were usually absent from both neurons and non-neuronal cells, including connective tissue cells, and were rare in Schwann cells of chickens aged less than five years. Inclusions were of two different types: filamentous bundles and granulofibrillar bodies. Individual nuclei contained one to three inclusions. Pseudo-inclusions, i.e. cytoplasmic pockets invaginated into the nuclei, were found more rarely and accompanied one or both types of 'true' inclusions. The possible significance of these findings in relation to ageing phenomena is discussed. It is concluded that intranuclear inclusions appear to be a consequence of nuclear/cellular activation and may be regarded as aggregates of previously dispersed intranuclear proteins.

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