Intermediate filament proteins in developing and adult human dorsal root and sympathetic ganglia

I. San José[1], G. P. Germanà[2], M.T. Vázquez, F.J. Naves[3], I. Esteban[3] and J.A. Vega[3]

1- Departamento de Anatomía Humana, Universidad de Valladolid, Spain
2- Dipartimento di Morfología, Biochimica, Fisiología e Produzioni animali, Università di Messina, Italy
3- Departamento de Morfología y Biología Celular, Universidad de Oviedo, Spain

SUMMARY

Neuronal maturation in the central nervous system, as well as in some cells deriving from neural crest, is accompanied by a swich in the expression of cytoskeletal intermediate filament proteins. Whether this occurs in humans and the exact timing of this change in human dorsal root and sympathetic ganglia are matters still open to debate. The present study was designed to analyze these issues in human embryos (estimated gestational age -e.g.a.- ranging between 6 and 12 weeks), as well as the possible co-expression of more than one intermediate filament protein in both embryos and adults. A panel of commercially available antibodies against vimentin, glial fibrillary acidic protein and neurofilament proteins was used. Glial fibrillary acidic protein was consistently absent in both developing and adult dorsal root or sympathetic ganglia. Conversely, embryonic neurons, satellite glial cells and Schwann cells displayed vimentin immunoreactivity. The number of vimentin immunoreactive neurons decreased progressively, and it was absent from neurons by 12 weeks e.g.a., while it persisted in satellite glial and Schwann cells. By adulthood, the pattern of distribution was identical. The occurrence of neurofilament proteins in peripheral neurons was a regular feature from early developmental stages to adulthood, and a time-dependent increase in the percentage of neurons containing phosphorylated neurofilaments was observed. The present results demonstrate that developing human dorsal root and sympathetic ganglion neurons co-express vimentin and neurofilaments for a short time, but that the intermediate filaments for mature neurons are neurofilaments. Our findings also show that co-expression or a switch in the expression of intermediate filament proteins do not occur in satellite glial cells or Schwann cells, which normally contain vimentin and not glial fibrillary acidic protein.

Key Words: Dorsal root ganglia – Sympathetic ganglia – Intermediate filament protein – Human

INTRODUCTION

The intermediate filament (IF) proteins form part of the cytoskeleton in eukaryotic cells (see for a review Fusch and Weber, 1994). They represent different but closely related proteins, most of them showing tissue specific distribution. During mammalian development, vimentin is the IF protein of undiffentiated neuroepithelial and neural crest derived cells, whereas their differentiated derivatives, neurons and glial cells, express neurofilament proteins (NFPs) or glial fibrillary acidic protein (GFAP), respectively (Cochard and Paulin, 1984; Lukas et al., 1991, 1993; Ophir and Lancet, 1988; Schwob et al., 1986). This switch in the neuronal expression of IF proteins has also been observed in peripheral sensory neurons, especially in the dorsal root ganglia (DRG; Lukas et al., 1991, 1993). Whether or not this also occurs in sympathetic ganglion (SG) neurons has not been reported.

Submitted: September 7, 2000
Accepted: December 23, 2000

Correspondence to:
José A. Vega. Departamento de Morfología y Biología Celular, Facultad de Medicina, C/ Julián Clavería, s/n. 33006-Oviedo, Spain
Phone and fax: +34 985 103671. E-mail: javega@correo.uniovi.es

In addition to neurons, the peripheral ganglia contain special glial cells called satellite glial cells (see Pannese, 1981). The IF proteins that form their cytoskeleton have been a matter of some debate (Schachner et al., 1974; Vega et al., 1989; Woodham et al., 1989), and it remains to be established whether they undergo developmental changes.

In the present study we used immunohistochemistry to analyze the cell distribution of vimentin, GFAP and NFPs in the developing and adult human DRG and sympathetic ganglia. During the developmental period analyzed, neurons first co-expressed vimentin and NFPs, after which they only contained the latter. Conversely, satellite glial cells and Schwann cells were always seen to express vimentin, but in no case GFAP.

Materials and Methods

Tissue treatment

Human lumbar DRG (n=8) were obtained during the removal of organs for transplantation from four adult male subjects (age range 26 to 53 years), who had died in traffic accidents. Lumbar paravertebral SG (n=12) were obtained during routine vascular surgery from 9 patients of both sexes (6 male and 3 female) of different ages (age range between 22 and 55 years). In both cases, samples were obtained in compliance with Spanish Laws, and after informed consent of the participants. DRG and SG from six human embryos were also used in the study. These were obtained in collaboration with obstetricians and were products of spontaneously (n=2) or artificially (n=4) interrupted pregnancies in compliance with Spanish Laws. The external features of these embryos were examined under a binocular dissecting microscope and the developmental stage of each embryo was determined. The estimated gestational ages (e.g.a.) were 6 (n=2), 8 (n=2), 9 and 12 weeks. Samples of adult human cerebellum and embryonic brain were also obtained and used as positive controls for the antigens investigated.

Specimens were collected in ice-cold physiological solution, fixed for 28 h with Bouin's fixative, and then dehydrated and embedded in paraffin. Sections were cut at 10 µm, mounted on gelatine-coated slides, deparaffinized, rehydrated and processed for PAP immunohistochemistry.

Immunohistochemistry

Sections were rinsed in HCl-Tris buffer (0.05 M, pH 7.5) containing 1% bovine serum albumin and 0.1% Triton X-100. Thereafter, endogenous peroxidase activity and non-specific binding were blocked, and the sections were incubated overnight with the primary antibodies in a humid chamber at 4ºC. The following mouse monoclonal antibodies (mAb) were used: (a) two mAbs directed against vimentin (clone 3B4, Boehringer-Mannheim, diluted 5 µg/ml; clone CKVNS from DPC purchased prediluted in a carrier protein preservative dilution); (b) two mAb directed against GFAP (clone G-A-5, Boehringer-Mannheim, diluted 5 µg/ml; clone CKGPS, from DPC purchased prediluted in a carrier protein preservative solution); (c) one mAb directed against an epitope of the phosphorylated 200 kDa NFP subunit (clone RT-97, Boehringer-Mannheim, diluted 5 µg/ml); (d) a rabbit polyclonal antibody against a common epitope of all neurofilament proteins (ref. NE4142, Sigma, diluted 1:400). Sections were then washed in the same buffer as above and incubated for 1 h at room temperature with peroxidase-labeled sheep anti-mouse IgG or sheep anti-rabbit IgG (Ammersahm, diluted 1:100). Finally, the antigen-antibody reaction was visualized as usual using DAB as a chromogen.

For control purposes, representative sections were processed in the same way with a non-immune mouse or rabbit serum, or with specifically absorbed sera (from DPC) instead of the primary antibodies. Under these conditions, no specific immunostaining was observed.

Quantitative analysis

The neuronal density and the number of neuronal perikarya showing vimentin and RT-97 IR were calculated from a ganglionic surface of 2 mm^2 using an automatic image analysis system (Servicio de Análisis de Imágenes, University of Oviedo). Measurements were made on five DRG ganglia (three sections per specimen 80 µm apart). Neuronal density was evaluated in sections stained with the NE4142 antibody, and the values obtained were considered as 100%. Data in the text and the table are expressed as means ± S.E. per mm^2.

Results

Embryos

The patterns of the distribution of immunoreactivity (IR) for NFPs and vimentin in DRG and SG were dependent on the gestational age, and on the antibody used (Fig. 1 A-C). Furthermore, GFAP was absent in the developing peripheral ganglia, but strongly GFAP-immunolabeled cells were seen in the central nervous system (data not shown).

Dorsal root ganglia

In the embryos of 6 weeks e.g.a., vimentin IR was restricted to the blood vessel walls and the connective septa dividing the DRG (Fig. 1D). At this time, vimentin IR was not detected in neu-

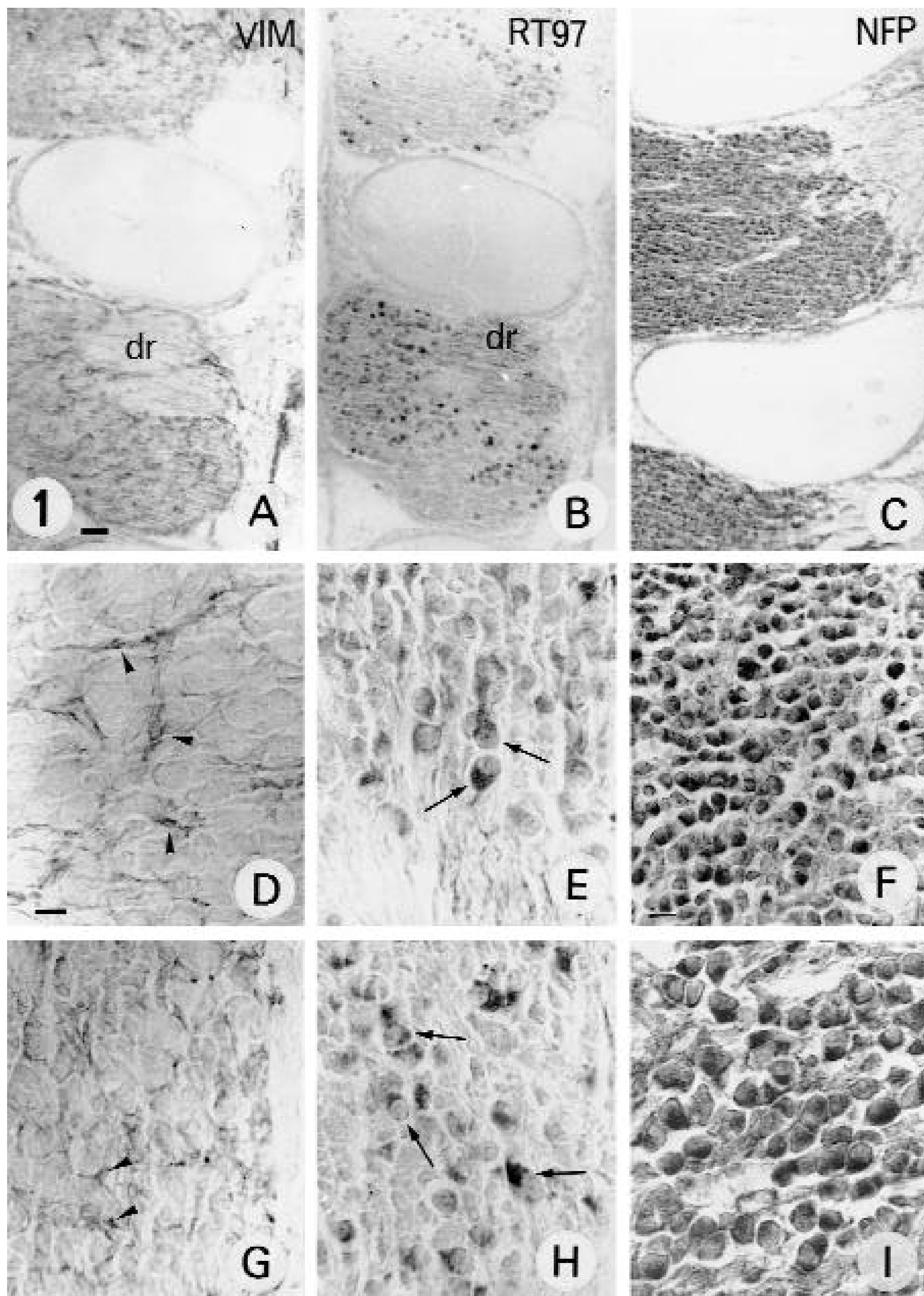

Fig. 1.- Immunohistochemical localization of vimentin and NFPs in developing human DRG neurons. Figures **A-C** and **G-I** correspond to embryos of 8 weeks e.g.a; figures **D-F** correspond to an embryo of 6 weeks e.g.a. Arrowheads in D and G indicate non-neuronal cells displaying vimentin IR with the antibody CKVNS; arrows in E and H indicate nerve cell bodies displaying RT-97 immunoreactivity. VIM: vimentin; RT-97: neurofilament proteins as revealed by clone RT-97; NFP: neurofilament proteins as demonstrated with the NE4142 antibody. Dr: dorsal root. Scale bar =70 µm for A-C; 20 µm for D-I.

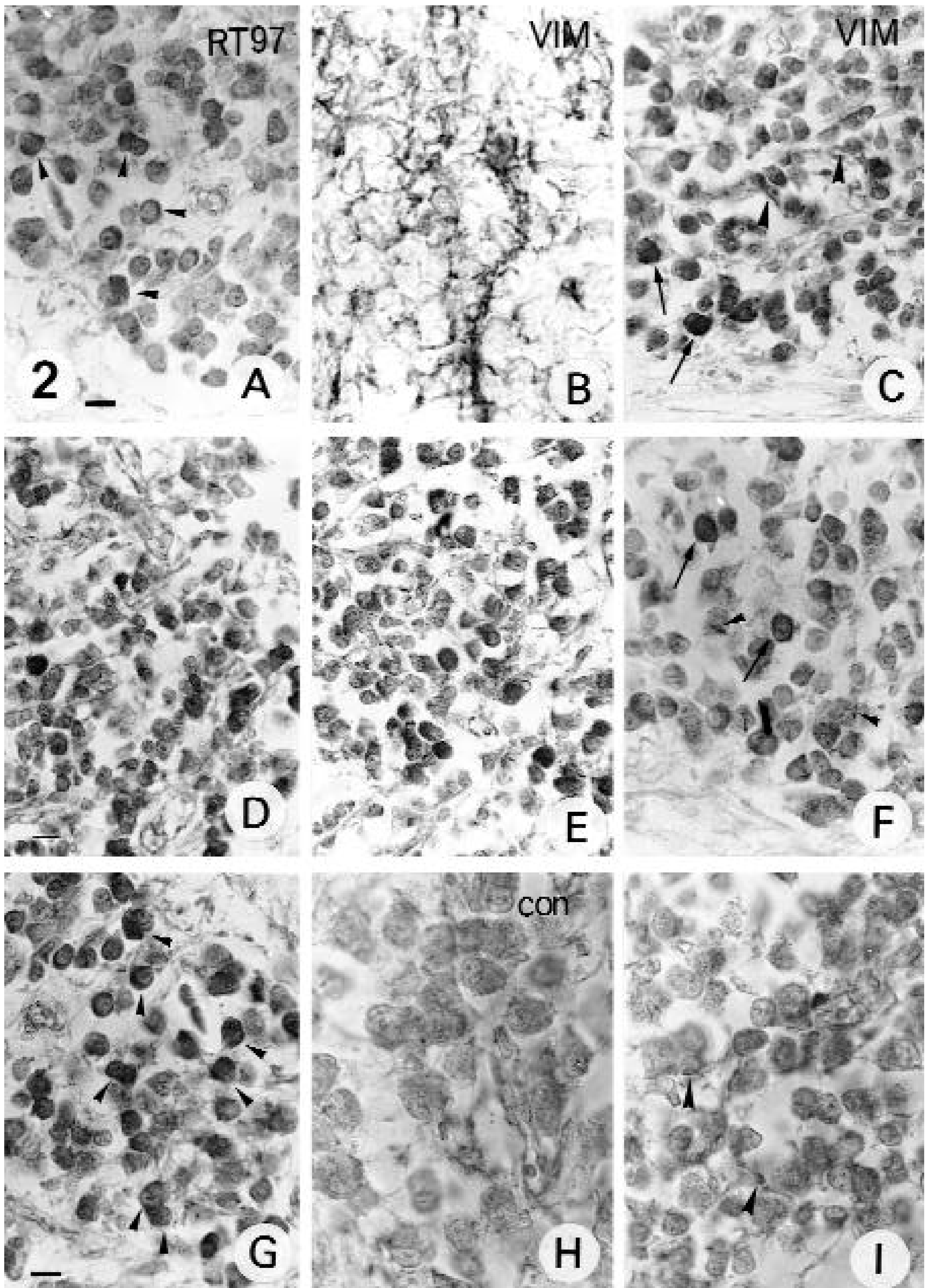

Fig. 2.- Immunohistochemical localization of RT-97 and vimentin IR in DRG of 8 (**A-C**), 9 (**D-F**) and 12 (**G-I**) weeks e.g.a. Arrowheads in A and D indicate immunoreactive perikarya. Arrows in C, F and I show vimentin immunoreactive perikarya, whereas arrowheads indicate non-neuronal cells. con: control section incubated with specifically pre-absorbed antibodies for vimentin. Scale bar = 20 µm.

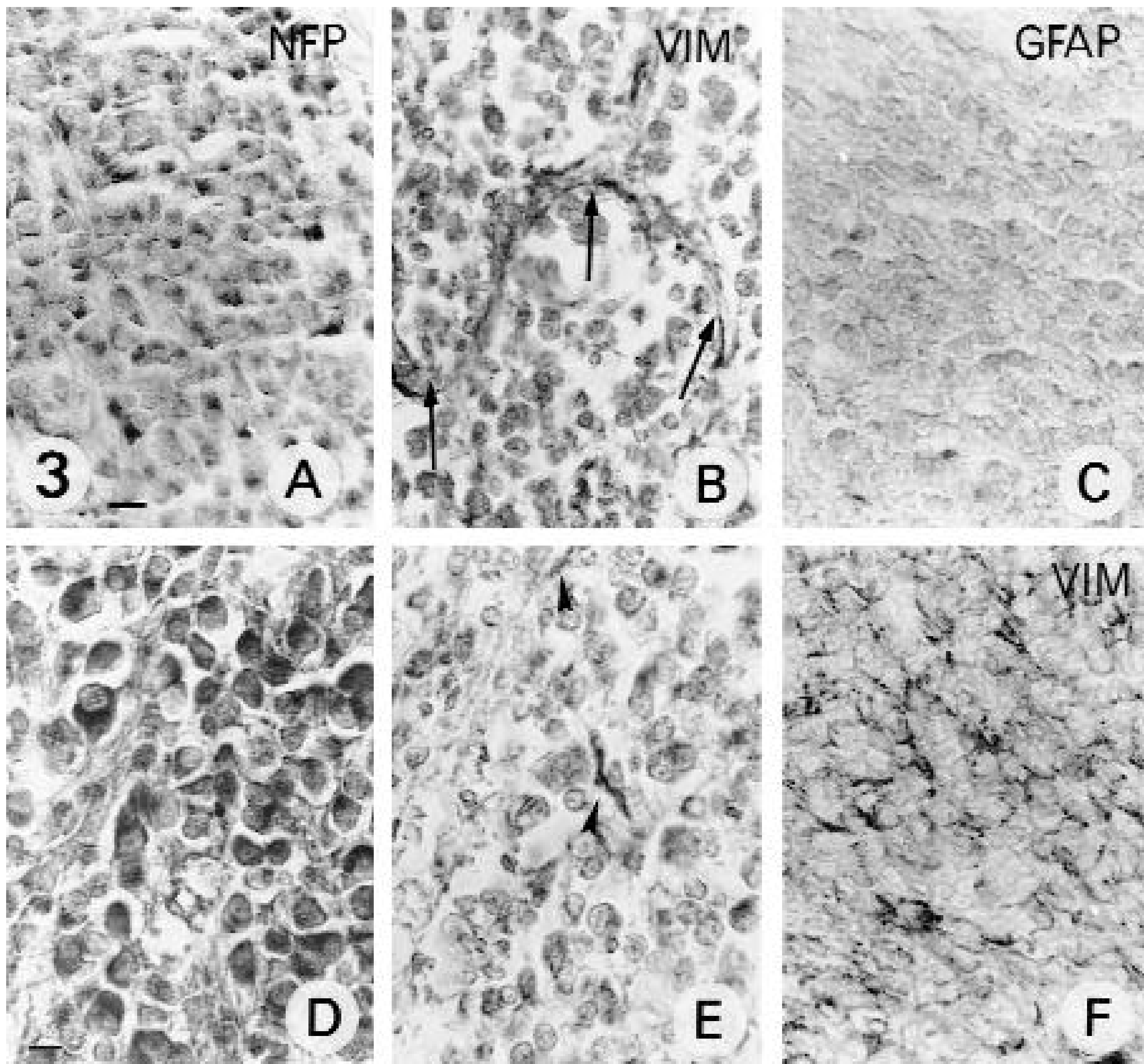

Fig. 3.- Immunohistochemical localization of NFPs (NFP, antibody NE4142, **A** and **D**), vimentin (clone 3B4, **B** and **E**; clone CKVNS, **F**) and GFAP(clone G-A-5, **C**) in the cervical SG of embryos of 8 (A-C) and 12 (D-F) weeks e.g.a. Arrows in B indicate a blood vessel showing intense IR for vimentin; arrowheads in E indicate non-neuronal cells displaying vimentin IR. Scale bar = 20 µm.

roblasts, and the occurrence of IR in developing satellite cells was not absolutely certain. Nevertheless, nearly all the neuronal perikarya displayed IR for NFPs (Fig. 1F) but only a subpopulation (approximately 11%) showed IR for phosphorylated NFPs (Fig. 1E). Two weeks later (8 weeks e.g.a.), all neuroblasts were immunoreactive for NFPs (Figs. 1C and 1I) and approximately 23% contained phosphorylated isoforms (Figs. 1B and 1H, 2A). Regarding vimentin, the results were different for the two antigens investigated. In fact, whereas clone CKVNS only labelled non-neuronal cells (Figs. 1A and 1G, 2B), clone 3B4 also stained a high percentage of neuroblasts (about 86%; Fig. 2C)

As development progressed, the most remarkable feature in the distribution of the antigens studied was the progressive increase in the percentage of RT-97 immunoreactive cells; this was 30% at 9 weeks and 42% at 12 weeks e.g.a. (Figs. 2D and G), respectively. Regarding neuronal vimentin IR, the reverse was seen, i.e., a progressive decrease in the percentage of nerve cell bodies (Figs. 2C, E, F, I), estimated to be around 60% at 9 weeks and 42% in some cases (2/9) at 12 weeks e.g.a. In the remaining ganglia (7/9) the percentage of labelled neuroblasts was very low (less than 5%), vimentin IR being restricted to non-neuronal cells (Fig.2I). No IR was observed in sections of DRG incubated with the primary antibody specifically preabsorbed for vimentin (Fig. 2H; Table 1).

Sympathetic ganglia

The above data described for developing DRG could also be applied to the cervical par-

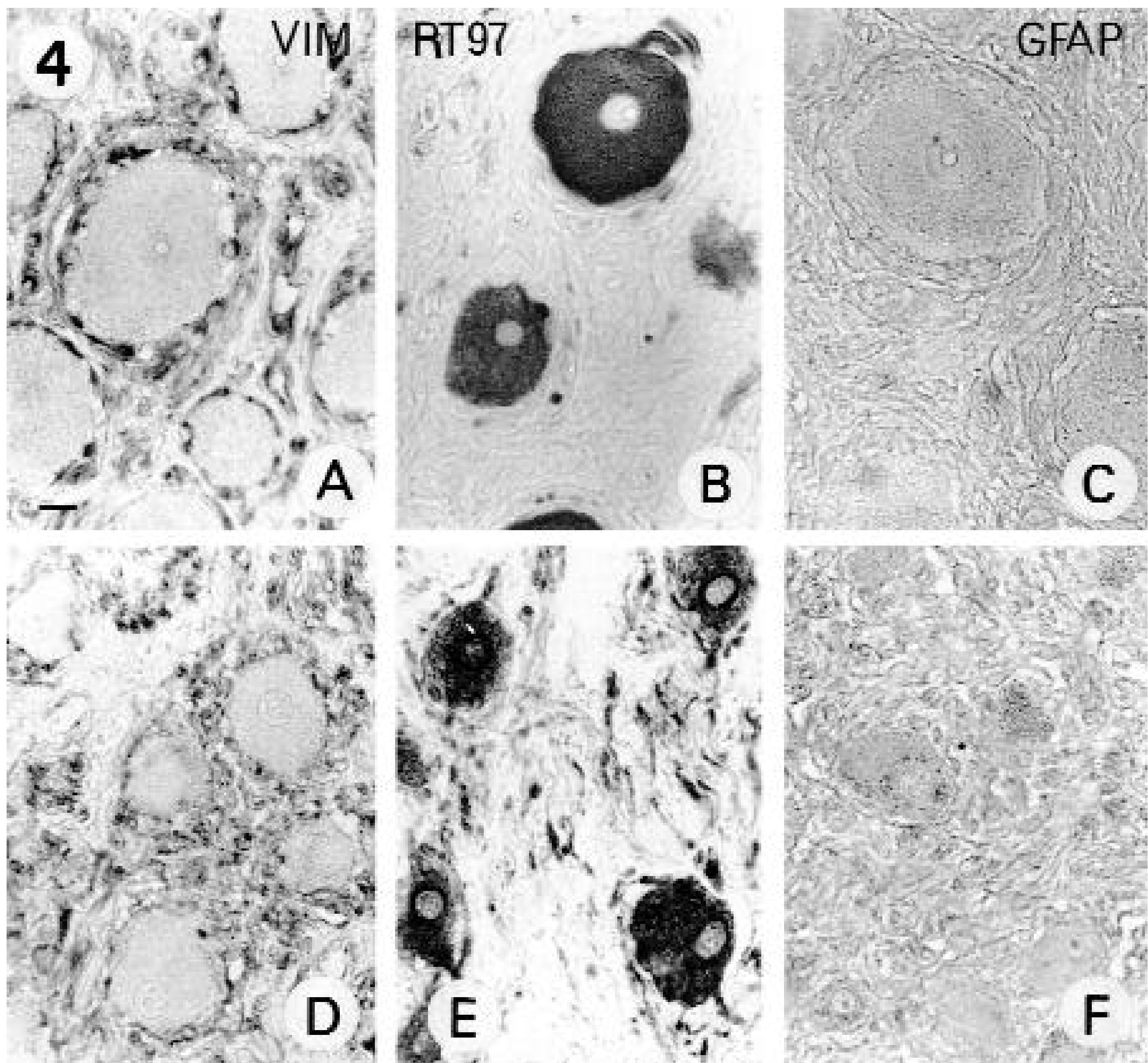

Fig. 4.- Immunohistochemical detection of vimentin (VIM, clone 3B4), neurofilament proteins (RT-97; clone RT-97) and GFAP(clone G-A-5) in adult human DRG (**A-C**, clone CKGPS and G-A-5, respectively) and paravertebral SG (**D-F**) . Scale bar = 20 µm.

avertebral SG. The NE4142 antibody labelled nearly all neuroblasts (Fig. 3D), as did RT-97 as well, although in 6 and 8 week e.g.a. embryos IR did not occupy the entire cytoplasm but instead was concentrated in one segment of the perikarya (Fig. 3 A). By contrast, the two antibodies used to detect vimentin showed a different pattern of immunostaining. Clone 3B4 was effective in demonstrating neuroblasts and non-neuronal cells including blood vessel walls (Fig. 3B). Conversely, clone CKVNS only revealed non-neuronal cells (Fig. 3F). In embryos of 8 and 9 weeks e.g.a., the percentages of developing neurons displaying vimentin IR were about 79% and 51%, respectively. In older embryos (12 weeks e.g.a.), only scattered neurons (9%) displayed vimentin IR while this was evident in all non-neuronal cells (Fig. 3E; Table 1). Finally, neither DRG nor SG displayed immunostaining for GFAP in any of the embryos studied (Fig. 3C).

In the brain, cerebellum, brain-stem and spinal cord of the studied embryos, antibodies against NFP labelled axons and also nerve cells. Vimentin and glial fibrillary antibodies pointed to astrocyte profiles and radial glia (data not shown).

Adults

In the adult human peripheral nervous system a segregation of cells was observed as regards the expression of vimentin and NFPs. The perikarya and processes of both DRG and SG neurons were strongly labelled for NFPs (Figs. 4B and 4E). Both satellite glial cells and Schwann cells were immunoreactive for vimentin (Figs. 4A and 4D) but lacked GFAP IR (Fig. 4C and 4F).

Table 1.- Percentage of nerve cell bodies displaying immunoreactivity for vimentin (clone 3B4) and phosphorylated neurofilament proteins (clone RT-97) in developing and adult human dorsal root and sympathetic ganglia.

Estimated gestational age	Vimentin	RT-97
Dorsal root ganglia		
6 weeks	negative	
8 weeks	86 ± 5	23 ± 2.2
9 weeks	60 ± 7.2	30 ± 1.3
12 weeks	4 ± 0.7*	42 ± 1.8
Adults	negative	100
Sympathetic ganglia		
6 weeks	not done	not done
8 weeks	79 ± 2.1	96 ± 2.3
9 weeks	51 ± 1.3	100
12 weeks	9 ± 0.9	100
Adults	negative	100

* in 2/9 ganglia 42 ± 3.3

Discussion

The presence of different types of IF proteins in neurons of the mammalian DRG and SG, including humans, is now well documented (for references see Naves et al., 1996; Vega et al., 1994). DRG neurons contain peripherin, α-internexin, and NFPs (Ferri et al., 1990; Naves et al., 1996; Vickers et al., 1991; Athlan et al., 1997), as well as vimentin during a short part of the development period (Lukas et al., 1991; 1993). The data available for SG have focused on NFPs (see Vega et al., 1994). The present study was aimed at investigating whether sympathetic and sensory peripheral neurons, as well as the satellite glial cells surrounding them, switch the expression of IF proteins during development to acquire the mature phenotype.

Previous studies in the central and peripheral nervous system have demonstrated that the earliest IF protein associated with neuronal development is nestin (or α-internexin) followed by vimentin. Finally, there is a further switch to NFPs. This sequence seems to vary slightly in different species (Escurat et al., 1990; Troy et al., 1990). According to this hypothesis, NFPs appear after terminal neuronal differentiation, followed by a gradual increase during late embryonic and early post-natal development (Ferri et al., 1990; Yachnis et al., 1992; for references, see also Xu et al., 1994). In addition, a series of papers have reported the occurrence of vimentin in both immature and mature neurons (Cochard and Paulin, 1984; Lukas et al., 1991; 1993; Ophir and Lancet, 1988; Schwob et al., 1986).

In our study we observed that developing human sensory and sympathetic neurons from embryos of 6 to 12 weeks of e.g.a. express IR for NFPs. Furthermore, we found a progressive increase in the percentage of DRG neurons displaying RT-97 IR (see table 1). This antibody labels an epitope of the phosphorylated 200 kDa NFP subunit (Lawson and Weddell, 1991) and has been extensively used to characterize sensory neuron subtypes in mammals (Lawson, 1992; Naves et al., 1996; Vega et al., 1994). Interestingly, phosphorylated NFPs were detected in the younger embryos of our series, and in those of Lukas and co-workers (Lukas et al., 1991; 1993). These data are at odds with those published by Keinert (1991) who had found this protein only after 25 weeks of e.g.a. Differences in tissue processing or in the sensitivity of the antibodies used might account for such discrepancies. Our data thus confirm previous studies in developing and adult sensory (Itoh et al., 1992; Naves et al., 1996; Vega et al., 1994) and sympathetic (Naves et al., 1996) peripheral neurons, demonstrating that phosphorylated NFPs are regularly present in neurons.

Concomitant to the expression of NFPs, both developing primary sensory and postganglionic sympathetic neurons also display vimentin IR. However, this labelling was detectable with only one of the two used antibodies used. Whether the other monoclonal antibody against vimentin was ineffective in labelling developing neurons remains obscure. It is probably directed against an epitope of the vimentin molecule that is not expressed in developing neurons or, alternatively, it may have been masked by the tissue treatment employed. Interestingly, the percentage of neurons displaying vimentin IR decreased with development. In our study, only a very small percentage of somata retained this ability at 12 weeks of e.g.a. whereas Lukas et al. (1991, 1993) reported some vimentin positive neurons in human embryos at 14 weeks of e.g.a. It therefore seems that the expression of vimentin by peripheral neurons is a premature event in development and that for a short time it coincides with the expression of NFP (between 6-12 weeks e.g.a. in our study; between 8-14 weeks e.g.a. in the studies by Lukas et al., 1991, 1993). Thus, our

data support the co-expression of two types of intermediate filament proteins during the development of human peripheral ganglia. Although no specific analysis or double immunolabeling studies were carried out, the quantitative data obtained here point to an inverse evolution in the percentage of RT-97 and 3B4 immunoreactive neurons (see table 1), especially in DRG. Whether these phenomena are linked should be clarified in future studies.

The second main goal of this study was to analyse the phenotype of satellite cells of the peripheral ganglia in expressing IF proteins. At ultrastructural level, filaments of about 10 nm thickness have been observed in satellite cells of both sensory and autonomic ganglia, as well as in Schwann cells (for references, see Schachner, 1974), but their exact protein composition is controversial (Dahl et al., 1992). In all embryos and adult peripheral ganglia (sensory and sympathetic) GFAP was never detected. These negative results for GFAP in human peripheral ganglia cannot be attributed to technical deficiencies since the protein was present in the central nervous system with a distribution matching that reported by Aquino et al. (1996) in human foetuses. By contrast, we consistently found that these cells express vimentin, whose density varied depending on the development stage, but not on the antibody used. These findings strongly support the absence of GFAP in satellite cells, as well as in Schwann cells, and lend credibility to the idea that the IF protein of these cells is vimentin. The results immunohistochemical studies carried out in different mammalian species are contradictory and the presence of both vimentin and GFAP (Bignami et al., 1989; Schachner et al., 1974; Woodham et al., 1989), vimentin alone (Vega et al., 1989) but not GFAP (Neuberger and Cornbrooks, 1989; Vega et al., 1989; Woodham et al., 1989) has been reported. The present results suggest that in developing and normal adults the satellite glial cells of the peripheral ganglia and Schwann cells express only vimentin, although in certain conditions they might express GFAP (Mancardi et al., 1991).

References

AQUINO DA, PADIN C, PEREZ JM, PENG D, LYMAN WD and CHIU FC (1996). Analysis of glial fibrillary acidic protein, neurofilament protein, actin and heat-shock proteins in human fetal brain during the second trimester. *Dev Brain Res*, 91: 1-10.

ATHLAN ES, SACHER MS and MUSHYNSKI WE (1997). Associations between intermediate filament proteins expressed in cultured dorsal root ganglion neurons. *J Neurosci Res*, 47: 300-310.

BIGNAMI A and DAHL D (1989). Vimentin-GFAP transition in primary dissociated cultures of rat embryo spinal cord. Int. *J Dev Neurosci*, 7: 343-357.

COCHARD P and PAULIN D (1984). Initial expression of neurofilaments and vimentin in the central and peripheral nervous system of the mouse embryos in vivo. *J Neurosci*, 4: 2080-2094.

DAHL D, CHI NH, MILES LE, NGUYEN BT and BIGNAMI A (1982). Glial fibrillary acidic (GFA) protein in Schwann cells: fact or artifact?. *J Histochem Cytochem*, 30: 912-918.

ESCURAT M, DJABALI K, GUMPEL M, FRANCOIS G and PORTIER MM (1990). Differential expression of two neuronal intermediate filament proteins, peripherin and the low-molecular- mass neurofilament protein (NF-L), during the development of rat. *J Neurosci*, 10: 764-784.

FERRI GL, SABANI A, ABELLI L, POLAK JM, DAHL D and PORTIER MM (1990). Neuronal intermediate filaments in rat dorsal root ganglia: differential distribution of peripherin and neurofilament protein immunoreactivity and effect of capsaicin. Brain Res, 515: 331-335.

FUSCH E and WEBER K (1994). Intermediate filaments: Structure, dynamics, functions and disease. *Ann Rev Biochem*, 63: 345-382.

ITOH T, SOBUE G, KEN E, MITSUMA T, TAKAHASHI A and TROJANOWSKI JQ (1992). Phosphorylated high molecular weight neurofilament protein in the peripheral motor, sensory and sympathetic neuronal perikarya: system-dependent, normal variations and changes in amyotropic lateral sclerosis and multiple system atrophy. *Acta Neuropathol* (Berl), 83: 240-245.

KEINERT R (1991). Immunohistochemical characterization of primitive neuroectodermal tumours and their possible relationship to the stepwise ontogenic development of the central nervous system. 1. Ontogenic studies. *Acta Neuropathol* (Berl), 82: 502-507.

LAWSON SN (1992). Morphological and biochemical cell types of sensory neurons. In Scott SA (ed). *Sensory neurons. Diversity, development, and plasticity* New York: Oxford University Press, pp 27-59.

LAWSON SN and WEDDELLS PJ (1991). Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons. *J Physiol*, 435: 41-63.

LUKAS Z, DRÁBER P, VIKLICKY V, DRÁBEROVÁ E and DOLEZEL S (1991). Expression of high-molecular-weight neurofilament protein and vimentin in human developing spinal cord and dorsal root ganglia. *Folia Biol*, (Praha) 37: 207-212.

LUKAS Z, DRÁBER P, BUCEK J, DRÁBEROVÁ E, VIKLICKY V and DOLEZEL S (1993). Expression of phosphorylated high molecular weight neurofilament protein (NF-H) and vimentin in human developing dorsal root ganglia and spinal cord. *Histochemistry,* 100: 495-502.

MANCARDI GL, CADONI A, TABATON M, SCHENONE A, ZICCA A, DE MARTINI I, BIANCHINI D, DAMIANI G and ZACCHEO D (1991). Schwann cell GFAP expression increases in axonal neuropathies. *J Neurol Sci*, 102: 177-183.

NAVES FJ, HUERTA JJ, GONZÁLEZ-SUÁREZ O, URDANGARAY N, ESTEBAN I, DEL VALLE ME and VEGA JA (1996). Distribution of immunoreactivity for cytoskeletal (microtubule, microtubule-associated, and neurofilament) proteins in adult human dorsal root ganglia. *Anat Rec*, 244: 246-256.

NEUBERGER TJ and CORNBROOKS CJ (1989). Transient modulation of Schwann cell antigens after peripheral nerve transection and subsequent regeneration. *J Neurocytol*, 18: 695-710.

OPHIR D and LANCET D (1988). Expression of intermediate filaments and desmoplakin in vertebrate olfactory mucosa. *Anat Rec*, 221: 754-760.

PANNESE E (1981). The satellite cells of sensory ganglia. *Cell Biol*, 65: 1-111.

SCHWOB JE, FARBER NE and GOTTLIEB DJ (1986). Neurons of olfactory epithelium in adult rat contain vimentin. *J Neurosci*, 6: 208-217.

SCHACHNER M, SOMER I and LAGENAUR C (1974). Expression of glial antigens C1 and M1 in the peripheral nervous system during development and regeneration. *Brain Res*, 316: 165-178.

TROY CM, BROWN K, GREENE LA and SHELANSKI ML (1990). Ontogeny of the neuronal intermediate filament protein, peripherin, in the mouse embryo. *Neuroscience*, 36: 217-237.

VEGA JA, HUMARA JM, NAVES FJ, ESTEBAN I and DEL VALLE ME (1994). Immunoreactivity for phosphorylated 200 kDa neurofilament subunit is heterogeneously expressed in human sympathetic and primary sensory neurons. *Anat Embryol*, 190: 453-459.

VEGA JA, RODRÍGUEZ C, MEDINA M, DEL VALLE-SOTO ME and HERNÁNDEZ LC (1989). Expression of cytoskeletal proteins in glial cells of dorsal root ganglia. *Cell Mol Biol*, 35: 635-641.

VICKERS JC, VITADELLO M, PARYSEK LM and COSTA M (1991). Complementary distribution of the neurofilament triplet and novel intermediate filament proteins in the autonomic and sensory nervous system of the guinea-pig. *J Chem Neuroanat*, 4: 259-270.

XU Z, DONG DLY and CLEVELAND DW (1994). Neuronal intermediate filaments: new progress on an old subject. *Curr Op Neurobiol*, 4: 655-661.

WOODHAM P, ANDERSON PN and TURMAINE M (1989). Satellite cells surrounding axotomised rat dorsal root ganglion cells increase expression of a GFAP-like protein. *Neurosci Lett*, 98: 8-12.

YACHNIS AT, RORKE LB, LEE VMY and TROJANOWSKI JQ (1992). Expression of neuronal and glial polypeptides during histogenesis of the human cerebellar cortex including observations on the dentate nucleus. *J Comp Neurol*, 334: 356-369.