The Apolipoprotein A-I Gene Is Actively Expressed in the Rapidly Myelinating Avian Peripheral Nerve

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Abstract. The expression of the apolipoprotein A-I (apo A-I) gene was investigated in the myelinating sciatic nerve. Hybridization analysis with an apo A-I cDNA probe obtained from a cDNA library of mRNA isolated from rapidly myelinating chick sciatic nerve indicated that apo A-I coding transcripts increase during development in the chick sciatic nerve in parallel with the increase of myelin lamellae. Substantial apo A-I–like immunoreactivity in chick sciatic nerve homogenates was detected by Western blotting. The amount of antigen increased from the 15-d embryonic stage to 1 d posthatch and then decreased. Two subcellular fractions corresponding to the cytoplasmic compartments were particularly enriched in apo A-I. apo A-I immunoreactivity was also found in highly purified myelin preparations. Immunohistochemical staining provided further evidence for the presence of apo A-I in the endoneurial compartment of the sciatic nerve. Electron microscopic examination of these fractions after negative staining showed the presence of spherical and disc-shaped particles resembling high density lipoproteins. The presence of apo A-I, cholesterol esters, phospholipids, and triacylglycerols in ultracentrifugal fractions corresponding to serum lipoproteins and the behavior of apo A-I on nondenaturing gradient gels implied that apo A-I was associated with lipid. Studies with short-term organ cultures of sciatic nerves from 1-d chicks strengthened the evidence for local synthesis and secretion of apo A-I and apo A-I–containing lipoproteins by this tissue. These results establish that the apo A-I gene is actively expressed in developing sciatic nerve during the period of rapid myelination. These findings support the hypothesis that apo A-I synthesized within the nerve participates in the local transport of lipids used in myelin biosynthesis.

THE myelin sheath of the peripheral nervous system (PNS)[1] is a vastly extended and modified plasma membrane of the Schwann cell (Geren, 1954). This membrane is wrapped around the nerve axon in a spiral fashion to form a tightly compacted, multilamellar structure. During development, Schwann cells accumulate along axons of growing peripheral nerves and proliferate until they completely engulf the axons (Speidel, 1964). Myelinogenesis is dependent on appropriate interactions of Schwann cells, axons, and extracellular matrix (Bunge, 1987). Apart from certain lipid precursors that may be obtained partially from the circulation or through axoplasmic transport from the neurons, the general consensus is that almost all myelin synthesis and assembly is carried out within the Schwann cell (Norton and Cammer, 1984). However, it is the nature of the axon that determines whether or not a given cell will produce myelin (Weinberg and Spencer, 1976; Bray et al., 1981). Little is known about the molecular details of the initiation, assembly, and maintenance of the myelin sheath.

The myelin membrane is composed of 70–80% lipids and 20–30% proteins. It has a relatively simple protein composition; a few proteins account for most of the total (Lees and Brostoff, 1984). The major proteins of the PNS myelin are PO and the basic proteins P1 and P2. On SDS-PAGE, the PO protein migrates at ~28–30 kD and constitutes ~50% of the total myelin protein. The polypeptide exhibits microheterogeneity caused by posttranslational glycosylation, acylation, sulfation, and phosphorylation and is induced specifically as a consequence of the interaction between Schwann cells and the myelinated type of axon (Brockes et al., 1981). Various morphological, physicochemical, and recent biochemical approaches (Lees and Brostoff, 1984; Kirschner et al., 1984; Lemke and Axel, 1985) indicate that the PO protein is an integral membrane protein of the PNS myelin. Recent experiments from our laboratory have demonstrated that the concentration of PO protein triples between day 16 and 18 of

1. *Abbreviations used in this paper*: apo A-I, apolipoprotein A-I; HDL, high density lipoprotein; LDL, low density lipoprotein; PNS, peripheral nervous system; S-2, supernatant 2; SO, supernatant of osmotically shocked myelin; VLDL, very low density lipoprotein.

embryonic development in the chicken (Nunn et al., 1987). This confirmed previous investigations by Uyemura et al. (1979): the ontogeny of PO protein parallels myelin deposition in the peripheral nerve and indicates the period of active myelination in the chick sciatic nerve is between the 14-d embryonic stage and ~5 d posthatch. As part of a long range investigation on myelination of the PNS, we prepared and screened a λgt 11 expression library of rapidly myelinating chick sciatic nerve in an attempt to obtain cDNA clones encoding the chick PO sequence. From this preparation several cDNA clones were isolated, which, upon sequencing, exhibited 100% identity to the 3′ coding and noncoding sequences of the chick apolipoprotein A-I (apo A-I) sequence recently reported by Rajavashisth et al. (1987). This unexpected, but nevertheless intriguing, result suggested that apo A-I might play a critical, hitherto unsuspected, role in the myelinogenesis of chick peripheral nerve.

Lipoproteins are specialized macromolecular complexes that serve as transporters of various lipids within the hydrophilic intra- or extracellular milieu (for review see Dolphin, 1985). apo A-I is the major protein component of plasma high density lipoprotein (HDL) in most animal species (Scanu et al., 1982). The polypeptide is synthesized in a variety of chicken tissues (Blue et al., 1982). Recently, three laboratories independently reported the isolation and characterization of full-length cDNA clones of chick apo A-I (Rajavashisth et al., 1987; Byrnes et al., 1987; Ferrari et al., 1987). The amino acid sequence deduced from the nucleotide sequence of these clones indicated that the chick protein is partially homologous to mammalian apo A-I and contains a number of tandem repeats of 11 and 22 residues. It has been suggested that these repeats can assume a largely amphipathic α-helical structure which might mediate binding to lipid molecules (Segrest et al., 1974). When the chick apo A-I cDNA clones were used as probes, apo A-I mRNA was detected in a variety of avian tissues, including liver, intestine, kidney, spleen, breast muscle, brain, and optic nerve (Ferrari et al., 1987; Rajavashisth et al., 1987; Byrnes et al., 1987). The level of these messages appeared to be developmentally regulated, particularly in liver, breast muscle, and intestine.

In this report, we present evidence that the apo A-I gene is actively expressed in the avian peripheral nerve. Expression of this gene is developmentally regulated, in parallel with the rapid phase of myelination. These findings support the hypothesis that apo A-I synthesized within the nerve participates in the local transport of lipids used in myelin biosynthesis.

Materials and Methods

Northern Blots

Sciatic nerves, from embryos or chicks (of various developmental stages), were dissected as described previously (Hu and Mezei, 1971). This tissue was immediately frozen in liquid nitrogen, and poly $(A)^+$ RNA was extracted by a modification of the hot phenol (LeBlanc and Mezei, 1985) or the guanidine isothiocyanate/hot phenol method (Maniatis et al., 1982) as described recently (LeBlanc et al., 1987). Poly $(A)^+$ RNA was purified by two successive cycles of binding to oligo(dT)-cellulose (Maniatis et al., 1982).

Northern analysis of total RNA was carried out on formaldehyde gels as described by Maniatis et al. (1982) with the following modifications. RNA (29–40 μg) was denatured at 65°C for 12 min in 50% formamide, 2.2 M formaldehyde, and 0.5× running buffer (1× running buffer is 20 mM 3-[*N*-morpholino]-propanesulfonic acid, 5 mM sodium acetate, 1 mM Na_2EDTA, pH 7) and quickly cooled on ice for 2 min. A 1.3% agarose gel containing 2.2 M formaldehyde, 1× running buffer, and 0.5 μg/ml ethidium bromide was prepared, and samples were electrophoresed in 1× running buffer at 40 V for 18 h. Transfer of formaldehyde-denatured RNA onto nitrocellulose was carried out as described by Maniatis et al., 1982. The 500-bp cDNA (pCSN-2) insert from the cDNA clone encoding the partial sequence of the chick apo A-I gene (nucleotide residues 530–959 according to Rajavashisth et al., 1987) was separated from the plasmid by Eco RI digestion of the recombinant plasmid DNA according to Maniatis et al. (1982), and the purified restriction fragments were labeled by random priming (Feinberg and Vogelstein, 1983) to a specific activity of $2.5–5 \times 10^8$ cpm/μg of DNA.

Slot Blots

2 μg of poly$(A)^+$RNA was diluted by 1:2 serial dilutions and applied through a nitrocellulose membrane fitted in a slot-blot apparatus as described recently (LeBlanc et al., 1987).

Subcellular Fractionation

Sciatic nerve tissue was dropped into liquid nitrogen upon dissection and ground with a mortar and pestle without allowing the tissue to thaw. The powder was then transferred into 12 vol of 0.85 M sucrose, 1 mM EDTA and homogenized for 2 min at 2,500 rpm in an all-glass tissue homogenizer (Kontes Glass Co., Vineland, NJ). The homogenate was subjected to discontinuous density gradient centrifugation to prepare crude myelin and related membranes according to a slightly modified procedure of Mezei and Verpoorte (1981) (Fig. 1). Typically 2.4 ml of tissue homogenate in the above solution was layered onto 1 ml of 1.2 M sucrose. Then 0.8 ml of 0.32 M sucrose was layered on top and the sample was subjected to centrifugation at 100,000 *g* for 120 min at 4°C. Six fractions (not osmotically shocked) were recovered from this gradient: supernatant 1 in 0.32 M sucrose; crude myelin layer at the 0.32/0.85 M sucrose interface; supernatant 2 (S-2) in 0.85 M sucrose; a very thin "lower band" fraction at the 0.85/1.2 M sucrose interface (corresponding to a similar band previously described for the chick sciatic nerve by Oulton and Mezei, 1976); supernatant 3 in 1.2 M sucrose; and a pellet. The crude myelin fraction was subjected to "osmotic shock" treatment by diluting it with 10 vol of ice-cold distilled water, followed by 10 strokes of hand homogenization in a homogenizer (Dounce; Kontes Glass Co.). The suspension was allowed to stand on ice for 15 min and centrifuged at 32,800 *g* for 30 min. The resulting supernatant solution (the SO fraction) was collected and further analyzed for the presence of apo A-I–containing lipoprotein particles. The myelin pellet was washed once with 20 vol of ice-cold distilled water followed by centrifugation at 32,800 *g* for 30 min and subjected to a second cycle of discontinuous sucrose gradient centrifugation as described above. The purified myelin fraction, which again formed a sharp band at the interface between the 0.85 and 0.32 M sucrose layers, was removed and washed five times with 20 vol of water as described above.

SDS-PAGE and Immunoblot Analysis

Sciatic nerve homogenates and subcellular fractions were prepared for electrophoresis by diluting them with a deoxycholate solution and protein solubilizer (4% β-mercaptoethanol, 0.25 M Tris, pH 8.6, 192 mM glycine, and 1% SDS) to obtain a final solution containing 0.05% deoxycholate and 75% protein solubilizer. These were boiled for 2 min and then cooled. Dye/glycerol (1.25% [wt/vol] bromophenol blue, 7.5% [vol/vol] glycerol) was added at 0.2% (vol/vol) before applying the sample on the electrophoresis gel. The electrophoresis, transfer of proteins to a nitrocellulose membrane, and immunoblotting were carried out as described by Nunn and Mezei (1984). The monospecific anti-chick plasma apo A-I antiserum (Blue et al., 1982) was used at a 1:1,000 dilution and anti-chick PO antibody (anti-chick apo A-I free as demonstrated by enzyme linked immunoabsorbant assay) at a 1:7,500 dilution. The second antibody was anti-rabbit IgG conjugated to alkaline phosphatase at 1:7,500 dilution.

Isolation of Lipoprotein Fractions

Subcellular fractions of chick sciatic nerve were separated into fractions corresponding to standard plasma lipoprotein fractions. The subcellular fractions were dialyzed overnight against a solution of 0.15 M NaCl, 0.001%

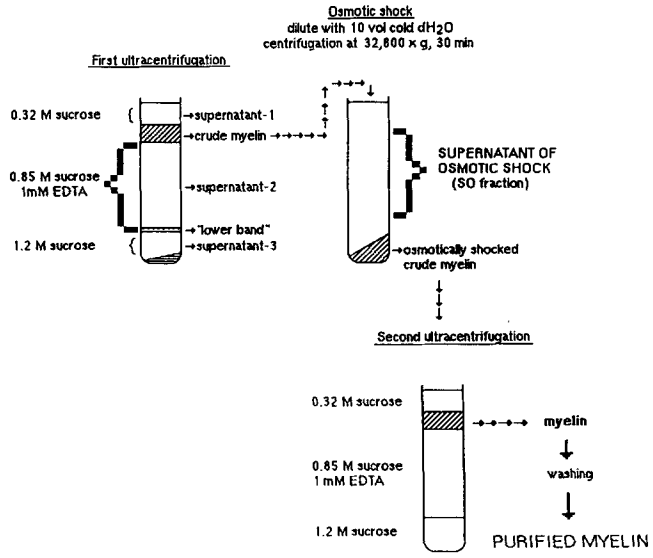

Figure 1. Scheme of subcellular fractionation used to prepare crude myelin and related membranes.

sodium azide, pH 7.6, and adjusted to a density of 1.21 g/ml with potassium bromide followed by ultracentrifugation (40,000 rpm in a rotor [50:3; Beckman Instruments, Inc., Fullerton, CA]) for 24 h. The supernatant and infranatant fractions were recovered for analysis or for additional fractionation. In some instances the $d < 1.21$ g/ml was dialyzed against a solution of 0.15 M NaCl containing 0.001% sodium azide (pH 7.6) and subjected to sequential ultracentrifugal flotation of lipoprotein fractions in a rotor (50:3; Beckman Instruments, Inc.) at $d = 1.006$ g/ml (18 h at 40,000 rpm), $d = 1.063$ g/ml (20 h at 40,000 rpm), and $d = 1.21$ g/ml (24 h at 40,000 rpm).

Analysis of Lipoprotein

Intact lipoproteins of $d < 1.21$ g/ml from sciatic nerve subcellular fractions were separated on nondenaturing 4–30% polyacrylamide gradient gels (Pharmacia Chem. Co., Montréal, Québec, Canada) by slight modification of the procedure of Nichols et al. (1983). The gels were preelectrophoresed for 20 min at 125 V before sample application. The samples were applied and electrophoresed for 20 min at 70 V followed by electrophoresis at 20°C for 20 h at 125 V. Gels were either stained with Coomassie brilliant blue or transferred to nitrocellulose for immunoblotting. The transfer was performed as previously (Nunn and Mezei, 1984), except that 0.02% SDS was added to the transfer buffer, and transfer was carried out at 4°C for 24 h at 30 V. The lipids in the lipoprotein fractions were quantitated by gas–liquid chromatography of total lipid profiles as described by Tam and Breckenridge (1983).

Negative Staining and Electron Microscopy

Aliquots of S-2 and SO subcellular fractions were mounted on copper grids coated with formvar and carbon. After 5 min, the excess liquid was removed and the sample was stained with 2% uranyl acetate for 20 s. The samples were viewed in an electron microscope (200; Philips Electronic Instruments, Inc., Mahwah, NJ) and photographed.

Detection of apo A-I and PO Protein In Situ

Sciatic nerves were removed from anesthetized 1-d chicks or 22-d rats after several minutes of fixation in situ with 4% (wt/vol) paraformaldehyde PBS (7.53 mM disodium hydrogen phosphate, 2.5 mM monosodium dihydrogen phosphate, and 145 mM sodium chloride, pH 7.1). The nerves were transferred to 4% formaldehyde in PBS and after an additional 4-h fixation they were embedded in paraffin by standard procedures. 4–6-μm-thick sections were immunostained according to the peroxidase-antiperoxidase method of Sternberger et al. (1970) with slight modifications. All sections were initially treated with 3% hydrogen peroxide in methanol to block endogenous peroxidase and 10% normal swine serum to reduce nonspecific binding of antiserum. Selected sections were treated with primary antiserum, rabbit anti-chick apo A-I at 1:100 dilution, and rabbit anti-chick PO IgG at 1:1,000 dilution. Control sections were treated with preimmune serum at 1:100 dilution. The sections were then incubated with swine anti-rabbit serum (1:30 dilution) and with rabbit peroxidase-antiperoxidase complex (Dimension Laboratories, Mississauga, Ontario, Canada). Color reaction was developed with 0.016% diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide. Sections were counterstained with Mayer's hematoxylin.

Labeling of Sciatic Nerves in Organ Culture

Sciatic nerves from 1-d chicks were dissected free of connective and other adherent tissue and placed immediately into sterile Krebs-Ringer solution containing 2 mg glucose/ml and previously saturated with 95% O_2/5% CO_2 at 2°C as described previously (Hu and Mezei, 1971). Aliquots of 0.25 g nerve tissue were incubated for 4 h at 37°C under an atmosphere of 95% O_2/5% CO_2 in 0.4 ml of the above medium containing 500 μCi [^{35}S]methionine (800 Ci/mmol L-[^{35}S]methionine, translation grade) and 25 μCi [^{14}C]amino acid mixture ([^{14}C]U-L-amino acid protein labeling mixture; DuPont of Canada Ltd., Montréal, Québec, Canada). After incubation, the medium was carefully removed by centrifugation and dialyzed against 2 liters of 0.02 M sodium phosphate, pH 7.4, 0.15 M sodium chloride containing 100 mg PMSF, 0.05 mg/ml NaN_3, and 1% (vol/vol) mercaptoethanol. The remaining nerve tissue was washed three times with ice-cold Krebs-Ringer solution followed by centrifugation at 600 *g* for 5 min. The tissue was then homogenized and centrifuged to prepare a high-speed supernatant according to Capony and Williams (1980). Another aliquot of sciatic nerve tissue, incubated and labeled as described above, was subjected to subcellular fractionation as described in a previous section to obtain purified myelin and related subcellular fractions.

Immunoprecipitation

Labeled tissue extracts, subcellular fractions, and medium were reacted with excess anti-chick apo A-I or anti-chick PO (anti-apo A-I-free) antibodies or preimmune serum in a double antibody procedure according to Blue et al. (1980) and LeBlanc et al. (1987). Protein A-Sepharose (Pharmacia Chem. Co.) was used as second antibody. Washed immunoprecipitates and original tissue extracts were fractionated by SDS-10% polyacrylamide slab gels as described previously (Nunn and Mezei, 1984). Radioactive proteins were visualized by fluorography (Bonner and Laskey, 1974). Low molecular weight protein standards (Pharmacia Chem. Co.) were routinely run on the gels for molecular weight calibration.

Results

Isolation and Sequence of a Chick apo A-I cDNA Clone

We prepared cDNA banks with mRNA isolated from sciatic nerves of 19-d chick embryos or 1-d posthatch chicks to obtain cDNA libraries containing maximum amounts of myelination-specific gene products. Immunoscreening of a λgt 11 expression cDNA library with polyclonal anti-chick PO antibody yielded a 200-bp recombinant cDNA. The sequence of the first isolates was homologous to the 3′ regions of chick apo A-I mRNA. This unexpected result was subsequently explained by demonstrating that the anti-chick PO IgG fraction contained a relatively high titer of anti-apo A-I antibodies. The apo A-I antibodies possibly arose from small amounts of apo A-I contaminating the purified PO antigen used to raise the polyclonal antibodies. Both PO protein and apo A-I have molecular masses of 28–30 kD. Screening of a pUC8 cDNA library from 1-d chick sciatic nerve with the 200-bp initial isolate yielded a 500-bp apo A-I cDNA clone that showed 100% identity to 291 nucleotides of the coding region corresponding to the carboxy terminus of the chick apo A-I protein and 186 bp of the 3′ noncoding region of the recently published chick apo A-I sequence from a chicken liver cDNA library (Rajavashisth et al., 1987).

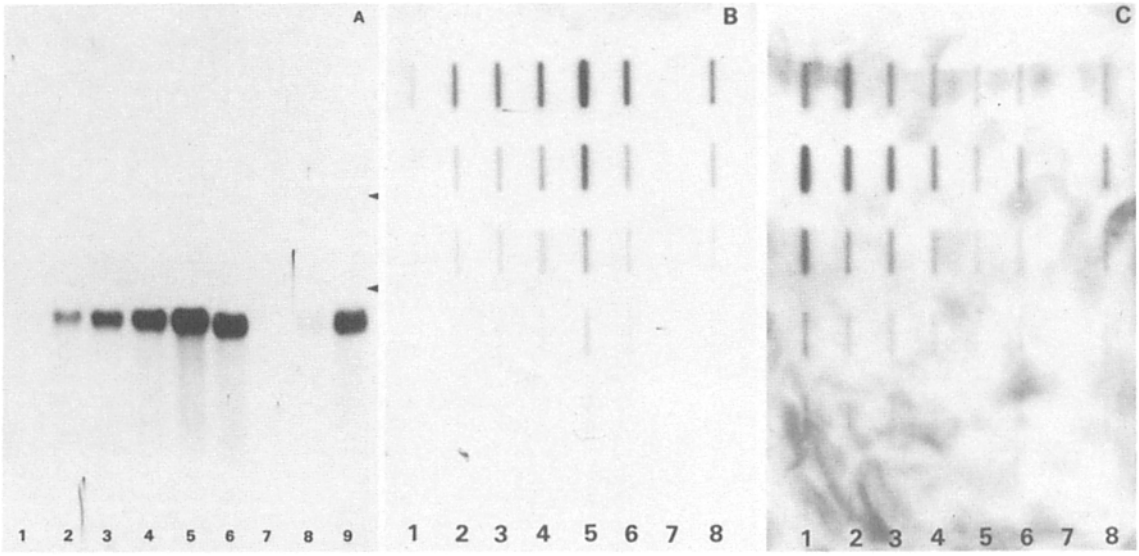

Figure 2. Northern and slot-blot analysis of mRNA from sciatic nerve, brain, and liver. (*A*) Northern blot analysis of total RNA preparations. The cDNA probe isolated from our 1-d-old chick sciatic nerve library and encoding nucleotide residues 530–959 of the cDNA clone reported by Rajavashisth et al. (1987) was hybridized to 29 (lanes *1* and *3–9*) and 40 μg (lane *2*) of total RNA. (Lanes *1–6*) Total RNA from sciatic nerves of 15-, 17-, and 19-d embryonic and 1-, 3-, and 7-d posthatch chicks, respectively; (lane *7*) wheat germ rRNA; (lane *8*) 19-d embryonic chick brain; (lane *9*) 19-d embryonic chick liver. The arrows indicate the position of 28 and 18 *S* rRNA markers on the gel. (*B*) Slot-blot analysis of poly(A)$^+$ RNA preparations. One in two serial dilutions of 2 μg poly(A)$^+$ RNA were applied on nitrocellulose filters and hybridized with the ^{32}P-labeled apo A-I cDNA probe as in *A*. (Lanes *1–6*) Poly (A)$^+$ RNA, respectively, from 15-, 17-, and 19-d embryonic and 1-, 3-, and 7-d posthatch sciatic nerve; (lane *7*) wheat germ rRNA; (lane *8*) 19-d embryonic liver RNA. (*C*) Slot-blot analysis of poly(A)$^+$ RNA preparations shown in *B*, stripped and rehybridized with ^{32}P-labeled histone variant H3.3 cDNA (Engel et al., 1982) as a control probe.

Developmental Expression of apo A-I mRNA in the Sciatic Nerve

To assess the size and abundance of apo A-I mRNA in the chick sciatic nerve, we used the 500-bp cDNA probe in Northern and slot-blot analysis of mRNA preparations (Fig. 2, *A* and *B*). Substantial amounts of apo A-I mRNA are found in the rapidly myelinating chick sciatic nerve (Fig. 2 *A*, lanes *2–6*). The message is barely detectable in 15-d embryonic nerve (Fig. 2 *A*, lane *1*) whereas no signal could be detected in nonmyelinated 12-d embryonic nerves (result not shown). The size of the transcripts (~1 kb), estimated from their migration relative to rRNA on the gels, is the same as that obtained from chick liver (lane *9*) or chick brain (lane *8*). As expected wheat germ rRNA does not show any hybridization with the cDNA probe (lane *7*). To obtain a better quantitation of the relative levels of apo A-I message from sciatic nerves of different developmental stages, we carried out slot-blot analysis of poly(A)$^+$RNA preparations. Fig. 2 *B* shows about an eightfold increase in the level of apo A-I mRNA in the sciatic nerve from the 15-d embryonic development to the 3-d posthatch stage (cf. lanes *1* and *5*). Myelin-specific genes and genes associated with the process of myelination are expected to increase during the phase of rapid myelination. Gene products not directly involved with myelination may show a different pattern. To demonstrate that the pattern of apo A-I gene expression does not merely reflect a general increase in transcriptional activity of the developing sciatic nerve we used the replication-independent histone variant H3.3 cDNA as a control probe (Engel et al., 1982). This gene has been used as control in other investigations of neural development (Vardimon et al., 1986). Fig. 2 *C* shows that levels of H3.3 coding transcripts are higher in the early stages of embryonic development than those of the posthatch animals. This is in contrast to the pattern observed for the apo A-I message. We may then conclude that the level of apo A-I transcripts increases during the active phase of myelination in the chick sciatic nerve (Uyemura et al., 1979; Nunn et al., 1987).

Immunochemical Detection and Subcellular Localization of apo A-I Protein in the Sciatic Nerve

To investigate whether the chick sciatic nerve contains substantial amounts of apo A-I protein, we fractionated sciatic nerve total protein extracts on SDS-polyacrylamide gels. Western blot analysis of these gels with monospecific anti-chick apo A-I antiserum (Blue et al., 1982) demonstrates immunostaining of a protein band in the sciatic nerve homogenates (Fig. 3, lanes *2–7*) and subcellular fractions (lanes *8–13*) that has the same mobility as purified chick serum HDL proteins (lane *1*). The high molecular weight protein bands in some of the preparations may represent aggregated forms of the apo A-I protein (Scanu et al., 1982). Densitometric comparison of the immunostained protein bands indicated maximum immunostaining per unit of total protein in nerve homogenates of 1-d-old chicks. The quantity of apo A-I-like immunoreactive material is ~3.5 times as great at 1-d posthatch as at the 15- or 17-d embryonic stage (lanes *2*, *3*, and *5*). The increase in the level of the translation products during nerve development is comparable, although not identical, with the developmental pattern of apo A-I transcripts shown by Northern analysis (Fig. 2 *A*).

If apo A-I has a role in the myelination process of the PNS,

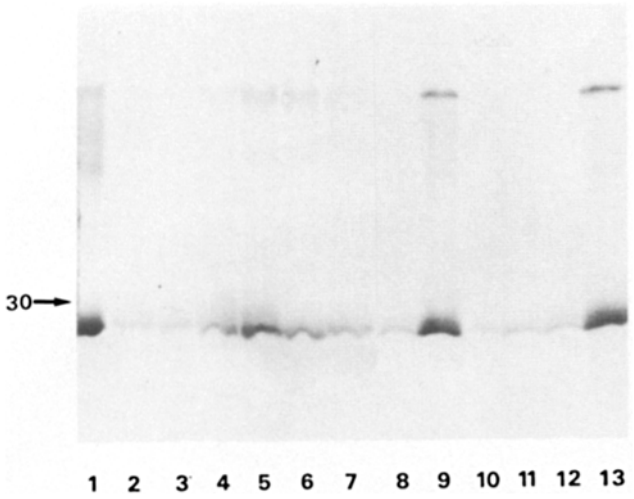

Figure 3. Detection of apo A-I protein in chick sciatic nerve homogenates and subcellular fractions. Proteins were fractionated on 10% SDS-polyacrylamide gels and electroblotted. The nitrocellulose sheets were immunostained with anti-chick apo A-I antiserum (1:1,000 dilution). Approximately 5 μg of total protein was applied in each lane, except in lane *1* which contained ~0.2 μg protein. (Lane *1*) Delipidated chick serum HDL proteins; (lanes *2–4*) sciatic nerve homogenates from 15-, 17-, and 19-d embryos; (lanes *5–7*) sciatic nerve homogenates from 1-, 3-, and 7-d posthatch chicks; (lane *8*) purified myelin; (lane *9*) SO fraction; (lane *10*) supernatant 3; (lane *11*) supernatant 1; (lane *12*) lower band; (lane *13*) S-2. The arrow and number on the side indicate the mobility and molecular weight of the 30K protein marker.

we would expect the protein to be present in cellular or subcellular fractions related to the myelin membrane. To explore the subcellular localization of apo A-I in the tissue, we subjected sciatic nerve homogenates to a subcellular fractionation procedure that yields various soluble, particulate, myelin-related, and highly purified myelin fractions (Oulton and Mezei, 1976; Lewis et al., 1984). apo A-I–like immunoreactivity was detected not only in the previously well-characterized and highly purified myelin fraction (Fig. 3, lane *8*), but also in other subcellular fractions obtained during the purification procedure (lanes *9–13*; for details of subcellular fractionation see Fig. 1). Two subcellular fractions, SO (Fig. 3, lane *9*) and S-2 (lane *13*), were enriched about twofold in apo A-I–like immunoreactivity when compared with the original homogenate (lane *5*). The SO fraction (lane 9) represents the supernatant fraction of crude myelin membranes subjected to osmotic shock treatment with cold distilled water; it therefore represents a myelin-derived fraction. Waehneldt (1978) suggested that osmotic shock treatment with cold water removes the hypoosmotically labile portions of the myelin superstructure, yielding a fraction enriched in tongue and loop regions and some shavings of lamellae initially involved in the compaction process of myelin formation. We speculate this fraction may also contain transitional forms of the Schwann cell membrane involved in myelin assembly. The S-2 fraction (lane *13*) was obtained at 0.85 M sucrose, between the crude myelin layer and a thin band above the 1.2 M sucrose cushion after the first discontinuous sucrose gradient cycle. This fraction probably corresponds to the soluble, cytoplasmic compartment of the sciatic nerve, but it also contains some membrane fragments derived from the myelin sheath (Lewis et al., 1984).

The resolution of the subcellular fractionation was tested by immunostaining the proteins of the subcellular fractions with anti-chick PO antibody. As expected and previously demonstrated (Nunn et al., 1987), PO protein, the major glycoprotein marker of the PNS myelin, was enriched in the highly purified myelin fraction (results not shown). A large proportion of apo A-I can be released from myelin by the osmotic shock treatment. However, a small but significant amount of apo A-I protein copurifies with highly purified myelin preparations. We confirmed the latter result by purifying myelin from 15- and 19-d embryonic and 7-d posthatch chicks and demonstrating the presence of significant amounts of apo A-I–like immunoreactivity in the preparations (result not shown). Our subcellular fractionation experiments thus indicate that apo A-I is present in the soluble portion of the sciatic nerve homogenates. Some of the polypeptide is also physically associated or entrapped within the myelin membranes.

Immunohistochemical Demonstration of apo A-I in the Chick Sciatic Nerve

We confirmed the presence of apo A-I protein within the sciatic nerve by immunohistochemical light microscopy of 4–6-μm sections of sciatic nerve preparations. Fig. 4, *A* and *B*, shows immunostaining of the endoneurial compartment of sciatic nerve with anti-apo A-I antibody. Higher magnification views (Fig. 4, *A'* and *B'*) indicate that some subcomponents of endoneurial cells and the extracellular matrix are also immunostained. As expected, myelin of chick sciatic nerve is heavily stained with the myelin-specific anti-chick PO antibody (*C* and *D*). Control experiments with preimmune serum (*E* and *F*) showed no reaction. Furthermore, specificity of antisera was demonstrated by carrying out the same type of experiments with rapidly myelinating, 21–23-d-old rat sciatic nerves. The results (not shown) demonstrated that anti-chick PO antibodies immunostained the myelin sheath, whereas anti-chick apo A-I showed no specific immunostaining in rat sciatic nerve sections. Therefore, it appears that apo A-I–like immunoreactivity is localized specifically in Schwann cells and extracellular matrix of the chick sciatic nerve.

Biosynthesis and Secretion of apo A-I Protein in Chick Sciatic Nerve

The series of experiments described above were designed to show the presence of apo A-I transcripts and translation products in the sciatic nerve. To demonstrate de novo synthesis of apo A-I and apo A-I–containing lipoprotein particles, sciatic nerves from 1-d chicks were incubated with a [^{35}S]methionine–[^{14}C]amino acid mixture for 4 h in organ culture. SDS-PAGE of the protein extracts followed by autoradiography revealed the presence of many radioactive proteins in the nerve (Fig. 5, lane *1*) and in the incubation medium (lane *7*). Newly synthesized apo A-I (lane *2*) and PO proteins (lane *3*) could be detected in the nerve by immunoprecipitation of these proteins from the nerve homogenate (lane *1*) with anti-chick apo A-I and anti-chick PO antibodies, respectively. The purified myelin fraction also contained

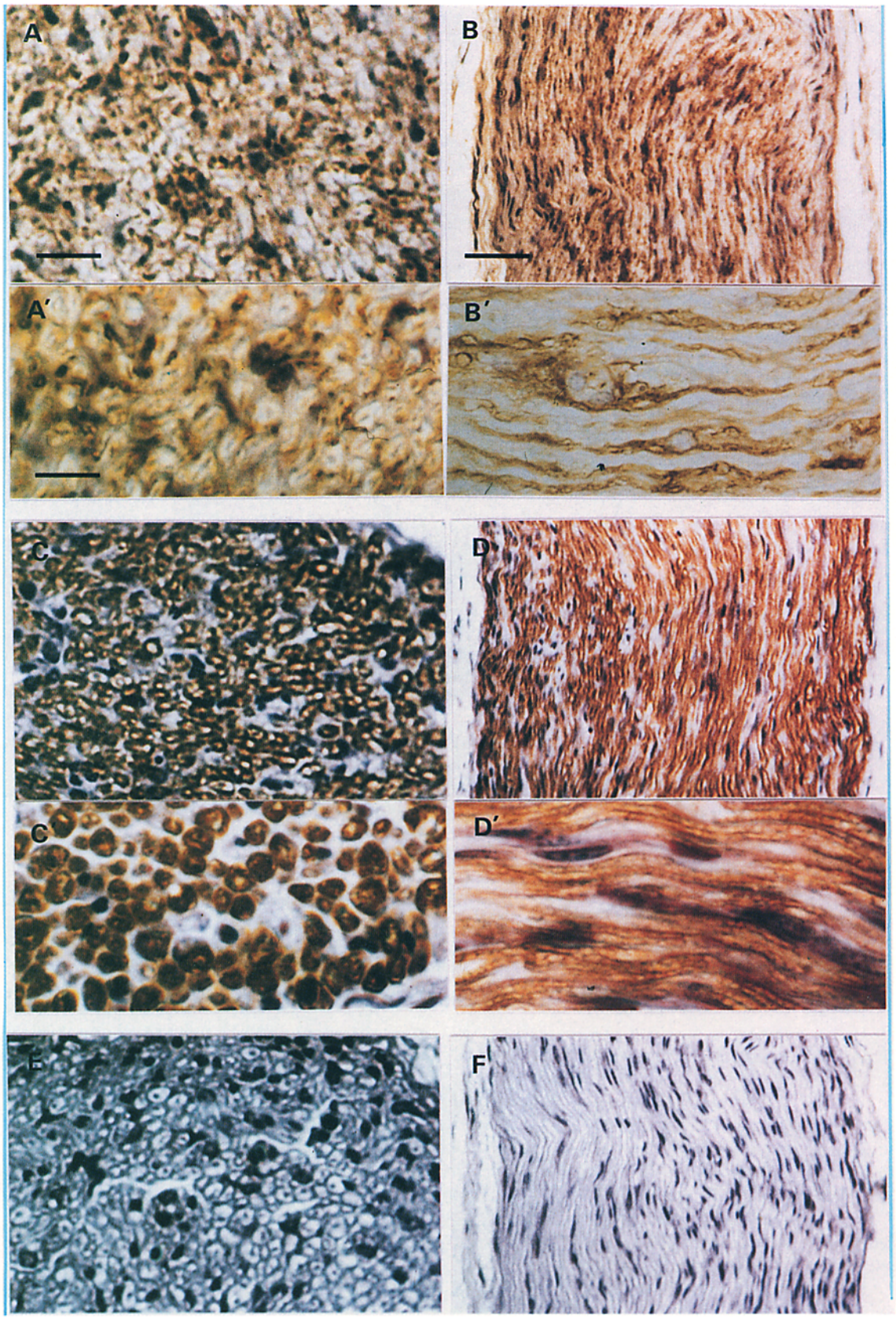

Figure 4. Immunocytochemical demonstration of apo A-I protein in chick sciatic nerve. Brown peroxidase reaction product marks the location of apo A-I protein or PO protein. (*A* and *B*) 4–6-μm sections of paraffin-embedded 1-d chick sciatic nerve incubated in anti-chick apo A-I antiserum (1:100 dilution); (*C* and *D*) consecutive sections of the same nerve, but incubated in anti-chick PO γ-globulin solution (1:1,000 dilution); (*E* and *F*) consecutive sections of the same nerve, but incubated in preimmune serum (1:100 dilution). Bars: (*A*, *C*, and *E*) 20 μm; (*B*, *D*, and *F*) 50 μm; (*A'–D'*) 12.5 μm.

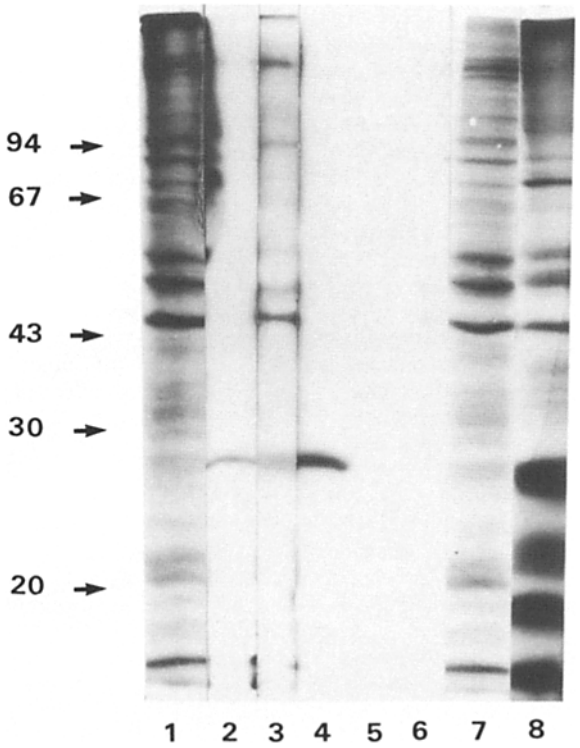

Figure 5. Biosynthesis and secretion of apo A-I protein in chick sciatic nerve. Sciatic nerves were incubated with a [^{35}S]methionine–[^{14}C]amino acid mixture in short-term organ culture. Tissue protein extracts and incubation medium were fractionated by SDS-PAGE, and newly synthesized proteins were detected by autoradiography. (Lanes *1* and *7*) Total proteins from the 226,000-*g* supernatant of homogenized nerves (3.1×10^5 cpm) and incubation medium (2.7×10^4 cpm), respectively; (lanes *2* and *4*) total proteins immunoprecipitated with anti-chick apo A-I antisera from the 226,000-*g* supernatant of nerve homogenates (1.7×10^6 cpm) and incubation medium (2.9×10^5 cpm), respectively; (lanes *3* and *5*) total proteins immunoprecipitated with anti-chick PO γ-globulins from the 226,000-*g* supernatant of nerve homogenates (1.7×10^6 cpm) and incubation medium (2.9×10^5 cpm), respectively; (lane *6*) total proteins immunoprecipitated with preimmune serum from the incubation medium (2.9×10^5 cpm); (lane *8*) total proteins from the purified myelin fraction. The arrows and numbers on the side indicate the mobility and molecular weights of low molecular weight protein markers (Pharmacia Chem. Co.). The gels were processed for fluorography (Bonner and Laskey, 1974), and lanes *2–6* were exposed on XOMAT-AR x-ray film (Eastman Kodak Co., Rochester, NY) for 25 h. Lanes *1*, *7*, and *8* were exposed for shorter or longer times to facilitate visualization of the individual radioactive protein bands.

newly synthesized myelin proteins, including the ~30-kD PO glycoprotein and the smaller basic proteins (lane *8*). Immunoprecipitation of radioactive apo A-I protein from the incubation medium (lane *4*) demonstrated the secretion of this apolipoprotein by the chick sciatic nerve. Although almost six times as much radioactive total protein was used for the immunoprecipitation of apo A-I and PO proteins from the high-speed supernatant of nerve homogenates as from the incubation medium, the intensity of the immunoprecipitated apo A-I band was twice as high in the incubation medium (cf. lanes *2* and *4*). This indicates that a large proportion of the newly synthesized apo A-I translation product was released from the nerves. As expected, the integral membrane glycoprotein PO was not secreted by the tissue (lane 5). This demonstrated the specificity of the secretion process. The specificity of the immunoprecipitation is shown by the absence of radioactive protein bands when immunoprecipitation was carried out with preimmune serum (lane *6*). Furthermore, the presence of a large excess of unlabeled antigens (HDL proteins and PO protein) eliminated the immunoprecipitation of newly synthesized radioactive antigens (results not shown; see Blue et al., 1982; LeBlanc and Mezei, 1985).

Detection of Lipoprotein-like Particles in Subcellular Fractions of Sciatic Nerve

Apolipoproteins normally occur in vivo in association with lipid to form lipoprotein particles. We deemed it important to determine whether apo A-I was associated with lipid in the sciatic nerve. Therefore, we subjected the subcellular fractions enriched in apo A-I to ultracentrifugation in KBr solution adjusted to a density of 1.21 g/ml. Ultracentrifugation yielded two major fractions: a fraction which floated on top of the 1.21 g/ml solution ($d < 1.21$ g/ml) and another with $d > 1.21$ g/ml. We analyzed these fractions by SDS–gel electrophoresis followed by immunoblotting and estimated that >60% of the total apo A-I and >80% of the total PO protein of the SO and S-2 fractions could be recovered in the "floating fraction" with $d < 1.21$ g/ml. These results indicated that a high proportion of the apo A-I protein (and, as expected, the PO protein) is associated with lipid within the sciatic nerve. We therefore further analyzed this floating fraction for the presence of lipoproteins and for lipid composition. Nondenaturing gradient gel electrophoresis that separates lipoprotein particles according to size (Nichols et al., 1983) was used. Coomassie blue staining of this gel detected two major protein bands in the S-2 and SO subfractions (Fig. 6 *A*, lanes *3* and *4*, respectively) that have mobilities similar but not identical to chick serum HDL particles (Fig. 6, lane *2*). The chick serum very low density lipoprotein (VLDL)–low density lipoprotein (LDL) fraction showed the presence of large particles barely entering the gel (Fig. 6 *A*, lane *1*). Control experiments with delipidated S-2 fraction (Fig. 6 *A*, lane *6*) showed a shift in mobility of the delipidated protein bands in a fashion similar to those of the delipidated chick serum HDL proteins (Fig. 6 *A*, lane *5*). Immunostaining of a blot of a duplicate gel with anti-chick apo A-I antibodies confirmed the presence of apo A-I–containing lipoprotein particles in the S-2 and SO subfractions (Fig. 6 *B*, lanes *3* and *4*, respectively) that have mobilities similar but not identical to chick serum HDL particles (Fig. 6 *B*, lane *1*). The specificity of immunostaining is further demonstrated by the absence of immunoreactivity in the chick serum VLDL–LDL fraction (Fig. 6 *B*, lane 2). However, these apo A-I–containing lipoproteins in S-2 and SO subcellular fractions were not immunoreactive with anti-PO or anti-apolipoprotein B (apo B) antisera when subjected to nondenaturing PAGE and Western blotting (results not shown). These experiments demonstrated that PO protein and apo B are not components of the HDL-like lipoprotein particles. The autoradiograms of immunoblots of S-2, SO, and incubation medium of sciatic nerves labeled with a [^{35}S]methionine and [^{14}C]amino acid mixture in organ culture also indicate that this tissue can synthesize (Fig. 6 *B*, lanes *7* and *8*) and secrete HDL-like lipoprotein particles into the extracellular space (Fig. 6 *B*, lane *6*).

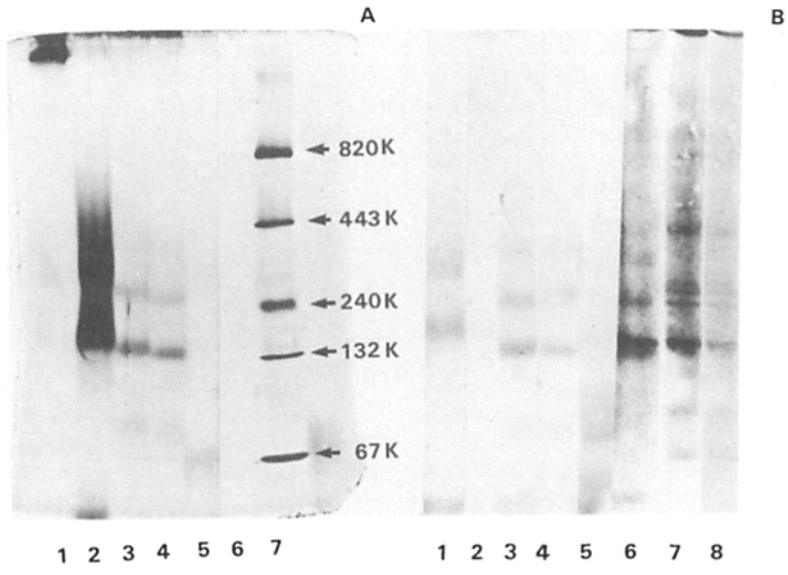

Figure 6. Nondenaturing polyacrylamide gradient gel electrophoresis and immunoblotting of subcellular fractions of chick sciatic nerve. The S-2 and SO subcellular fractions, serum HDL, and VLDL–LDL fractions isolated after potassium bromide ultracentrifugation and the incubation medium and S-2 and SO subcellular fractions of 1-d chick sciatic nerves labeled with a [^{35}S]methionine–[^{14}C]-amino acid mixture in organ culture were analyzed on 4–30% nondenaturing polyacrylamide gels. The proteins on the gels were stained with Coomassie brilliant blue (*A*) or transferred to nitrocellulose for immunoblotting (*B*). The blots were immunostained with anti–chick apo A-I antiserum (1:1,000 dilution) and the stained blots subjected to autoradiography to detect newly synthesized endogenous and secreted apo A-I proteins. In *A*: (lane *1*) 1-d chick serum VLDL–LDL; (lane *2*) 1-d chick serum HDL; (lane *3*) S-2 fraction; (lane *4*) SO fraction; (lane *5*) delipidated 1-d chick serum HDL proteins; (lane *6*) delipidated S-2 fraction; (lane *7*) high molecular weight protein standards (Pharmacia Chem. Co.). The arrows and numbers on the side indicate the mobility and molecular weights of the protein markers. In *B*: (lane *1*) chick serum HDL, (lane *2*) chick serum VLDL–LDL fraction; (lane *3*) S-2 fraction; (lane *4*) SO fraction; (lane *5*) delipidated chick serum HDL proteins; (lanes *6–8*) autoradiograms of immunoblots of the incubation medium and S-2 and SO subcellular fractions of 1-d chick sciatic nerves labeled with [^{35}S]methionine–[^{14}C]amino acid mixture for 4 h in organ culture. X-ray films of immunoblot lanes *6–8* were exposed for various time periods to facilitate visualization of the individual radioactive protein bands.

To investigate further the chemical nature of the lipids associated with the lipoprotein particles in the sciatic nerve, we analyzed the lipids in the S-2 and SO floating fractions ($d <$ 1.21 g/ml). Table I shows that both of these subcellular fractions contained cholesteryl esters and triacylglycerols, suggesting the presence of neutral core lipoprotein-like particles. The supernatant fractions (d = 1.21 g/ml) from S-2 and SO were further resolved into subfractions corresponding to serum VLDL ($d <$ 1.006 g/ml), LDL (d = 1.006–1.063 g/ml), and HDL (d = 1.063–1.21 g/ml) as shown in Table I. Very small amounts of lipid were present in the VLDL fraction, clearly indicating that the majority of cholesteryl ester and triacylglycerol was not associated with nondefined floating fat. There were substantial amounts of all lipid classes in the LDL- and HDL-like fractions. Although most of the lipid in S-2 was present in the HDL-like fraction, in the SO fraction the lipid in the LDL-like fraction was the most abundant.

All subfractions of S-2 had substantial amounts of cholesteryl ester and triacylglycerol which is characteristic of neutral core lipoproteins for these density classes. However, in SO the majority of the lipid mass in the LDL- and HDL-like fractions was cholesterol and phospholipid. All fractions contained higher proportions of cholesterol than serum lipoproteins. In view of the substantial amounts of PO protein it is probable that there are some myelin membrane fragments in these lipoprotein-like fractions. However, discoidal HDL particles (Pitas et al., 1987), as well as HDL

Table I. Composition of Subfractions of S-2 and SO Corresponding to Standard Lipoprotein Fractions

	Subfraction of peripheral nerve*									
	S-2				SO				Plasma‡	
	Total	$d <$ 1.006 g/ml	d = 1.006–1.063 g/ml	d = 1.063–1.21 g/ml	Total	$d <$ 1.006 g/ml	d = 1.006–1.063 g/ml	d = 1.063–1.21 g/ml	VLDL + LDL	HDL
Lipid mass distribution (% of total)	100	3.3	18.4	78.3	100	6.0	63.2	30.8	–	–
Composition (wt%)§										
C	21.9 ± 2.2	10.4	20.7	19.9	22.4 ± 7.1	4.9	17.8	32.8	12.3	6.3
CE	23.3 ± 5.7	43.5	29.4	19.9	14.3 ± 7.9	25.1	5.8	3.4	47.8	39.2
PL	44.2 ± 7.4	21.9	34.2	50.9	47.8 ± 8.8	26.8	57.5	52.8	34.5	51.3
TG	10.6 ± 2.9	24.2	15.7	5.2	15.9 ± 2.5	43.2	18.9	11.1	5.4	3.2

* Preparations of S-2 and SO were adjusted to d = 1.21 g/ml and centrifuged for 24 h in a rotor (50.3; Beckman Instruments, Inc.) at 40,000 rpm to yield a supernatant containing the total membrane plus lipoprotein fraction which contained 98% of the lipid. The supernatant fraction was dialyzed against NaCl solution (d = 1.006 g/ml). Standard lipoprotein fractions were isolated as described in the text.
‡ Serum lipoproteins were isolated from 1-d chicks as described in text.
§ *C*, Cholesterol; *CE*, cholesteryl ester; *PL*, phospholipid; and *TG*, triacylglycerol.

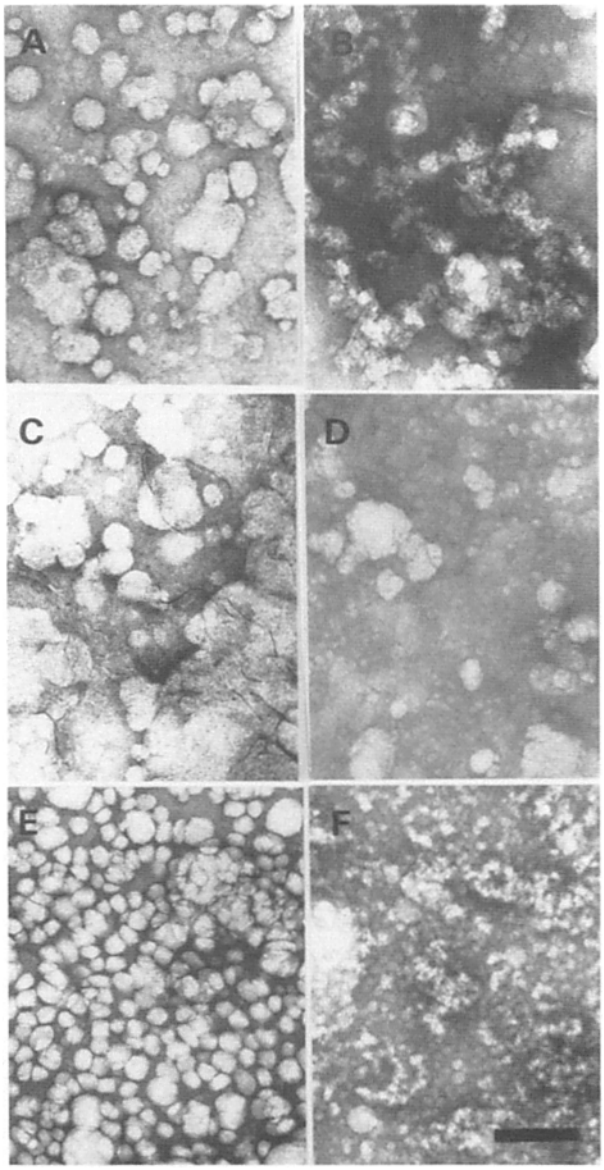

Figure 7. Electronmicrographs of negatively stained preparations of sciatic nerve S-2 and SO subcellular fractions and chick serum lipoproteins. Negatively stained particles from lipoprotein fractions of 1-d-old chick sciatic nerve S-2 (*A* and *B*); SO subcellular fractions (*C* and *D*); and 1-d chick serum (*E* and *F*). (*A* and *C*) The particles had d = 1.006–1.063 g/ml (LDL-like); (*B* and *D*) d = 1.063–1.21 g/ml (HDL-like); (*E*) $d < 1.063$ g/ml (serum VLDL–LDL fraction); and (*F*) d = 1.063–1.21 g/ml (serum HDL).

from cerebrospinal fluid, contain higher proportions of cholesterol than normal plasma HDL. Furthermore, the high amount of lipid in the LDL-like fraction may be due to large apo A-I–containing lipoproteins similar to those described for peripheral lymph (Sloop et al., 1987).

Electronmicroscopic analysis further supported the results shown in Table I. Electronmicrographs of the negatively stained LDL-like fractions (d = 1.006–1.063 g/ml) from the S-2 and SO subcellular fractions show the presence of vesicular structures and spherical particles (Fig. 7, *A* and *C*) that probably represent myelin fragments and resemble the VLDL–LDL fraction of 1-d-old chick serum (*E*). The HDL-like fractions (d = 1.063–1.21) from the S-2 and SO subcellular fractions contain spherical (*B* and *D*) or disc-shaped particles that are also assembled into stacks (*B*), similar to the HDL particles from chick serum (*F*). The mean diameters of spherical particles from the HDL-like fractions of the S-2 and SO subcellular fractions were ~10 nm. This result is comparable with that reported for the spherical lipoprotein particles of human or canine cerebrospinal fluid and chick HDL fractions (Pitas et al., 1987; Banerjee and Redman, 1983). These data are thus consistent with the presence of apo A-I–like immunoreactivity in the chick subcellular fractions that are associated with lipids. Some of the physicochemical properties of these lipoprotein-like particles resemble those of lipoproteins in the cerebrospinal fluid and peripheral lymph observed previously by other investigators (Pitas et al., 1987; Sloop et al., 1987).

Discussion

Our investigations indicate that transcripts and translation products of the apo A-I gene are present in the avian peripheral nerve. Moreover, the expression of this gene appears to be developmentally regulated during a period of nerve development that coincides with the phase of most rapid myelination of peripheral axons (Uyemura et al., 1979; Nunn et al., 1987). These experiments disclose a new and hitherto unsuspected role for apo A-I in the myelination process of the avian PNS. Several lines of evidence support this role.

We isolated a cDNA encoding the partial sequence of apo A-I from cDNA libraries of rapidly myelinating chick sciatic nerve. Positive clones in these libraries arose at a frequency of 0.3%, indicating that apo A-I coding transcripts were relatively abundant in the tissue. A relatively high abundance of the apo A-I transcripts in the sciatic nerve was also confirmed by Northern analysis of the mRNA populations (Fig. 2 *A*), providing evidence for the developmental regulation of the expression of the apo A-I gene. The level of mRNA is very low in the 15-d embryonic nerve, where the number of myelin lamellae around the growing axons is very small. Thereafter, the amount of apo A-I message increases rapidly up to 3-d posthatch, in parallel with the rapid deposition of myelin in the sciatic nerve. Recently, a similar developmental study of apo A-I mRNA levels in various tissues was described in skeletal muscle, where this mRNA species is present at a high level only immediately after hatching (Rajavashisth et al., 1987; Ferrari et al., 1987). In contrast, the low level of chick brain apo A-I mRNA does not appear to vary with age (Ferrari et al., 1987). It has been suggested that activation of apo A-I synthesis in muscle might be caused by the innervating motor neurons or by metabolic stimuli such as changes in lipid composition and concentration which occur at the time of hatching (Schackelford and Lebherz, 1983). Similar types of mechanisms might be operating in the sciatic nerve.

The developmental regulation of expression of the apo A-I gene is also demonstrated by our immunoblotting experiments (Fig. 3). apo A-I protein is detectable in nerve homogenates of 15-d embryos, and the level increases up to 1-d posthatch. The accumulation of apo A-I protein appears to precede that of its transcripts. Although the apolipoprotein does not appear to be enriched in myelin, a portion remains tightly associated with myelin even after extensive purification (Fig. 3). Our immunohistochemical analysis confirmed the presence of apo A-I–like immunoreactivity in endoneu-

rial cells and possibly in the extracellular matrix between the endoneurial cells.

Our studies with organ cultures of sciatic nerves from 1-d chicks strengthen the evidence for local synthesis and secretion of apo A-I by this tissue. Newly synthesized apo A-I was identified by immunoprecipitation with antibody specific to apo A-I of chick serum (Fig. 5). The immunoprecipitated apo A-I synthesized by the sciatic nerve had the same relative mobility as apo A-I from chick serum. The newly synthesized apo A-I was secreted by the sciatic nerve in a form associated with lipid (Fig. 6). Similar results were reported for chick muscle cells by Blue et al. (1982). As expected, the synthesis, but not the secretion, of the major peripheral myelin protein, PO, also occurred under these conditions.

Our working hypothesis that the nonneuronal cells of the avian sciatic nerve synthesize and secrete apo A-I associated with specific lipids was confirmed by showing the presence of HDL-like particles in two subcellular fractions that probably represent the cytoplasmic compartment actively involved in the synthesis and assembly of myelin precursors. The flotation properties ($d < 1.21$ g/ml) and the electromicroscopic appearance of the negatively stained particles resembled those of HDL particles from chick serum (Fig. 7). The presence of cholesterol esters and triacylglycerols in this fraction (Table I) and the absence of these components in highly purified myelin (results not shown) further confirmed the association of apo A-I with classes of lipids normally found in lipoprotein particles. One important difference is the high proportion of free cholesterol in the lipid extracts of chick sciatic nerve cytoplasmic fractions when compared with plasma HDL cholesterol (Scanu et al., 1982). Interestingly, the lipid composition of the sciatic nerve lipoprotein fraction is similar to the free cholesterol enriched particles found in mammalian cerebrospinal fluid that are believed to be assembled locally (Pitas et al., 1987). Our data are consistent with the notion that the sciatic nerve lipoprotein particles are assembled locally in the nerve and are not derived from plasma. The possibility remains, however, that the high free cholesterol content of these subcellular fractions was partially due to contamination with myelin fragments resulting from the freezing–thawing and homogenization procedures used to prepare the subcellular fractions. Experiments are in progress to further purify and characterize the lipoprotein particles of the chick sciatic nerve.

Our results, indicating that the apo A-I gene is actively expressed in the peripheral nerve during the time of rapid myelination, raise questions as to the role of apo A-I in myelination. A number of laboratories reported recently that apolipoprotein E (apo E) plays an important role in lipid homeostasis, development, demyelination, and nerve repair in the mammalian nervous system (Dawson et al., 1986; Basheeruddin et al., 1987; Snipes et al., 1987; Ignatius et al., 1987). apo E has not been detected in avian lipoproteins, but apo A-I is synthesized in a variety of chicken tissues (Blue et al., 1982), including the optic nerve in which apo A-I synthesis is increased fivefold during Wallerian degeneration (Dawson et al., 1986). These results led to the proposal that apo A-I is the avian counterpart to mammalian apo E and the two proteins may perform functions common to both classes of animals in a variety of peripheral tissues (Dawson et al., 1986). Two major functions have been proposed for these lipoproteins: the redistribution of lipids among cells of different organs and the redistribution of lipids among cells within an organ or tissue (Blue et al., 1982, 1983; Dawson et al., 1986; Mahley, 1988). Recently Mahley (1988) emphasized that other functions of apo E, unrelated to lipid transport, are becoming known, particularly those involving modulation of cell growth and differentiation.

One of the most striking examples of cell–cell interactions and differentiation of a cell type is the myelination of central and peripheral axons. The formation of myelin imposes an enormous burden on the metabolism of the myelinating Schwann cell. It has been estimated that on the average, each cell makes an amount of myelin per day that is more than three times its own weight (Norton and Cammer, 1984). The avian myelin sheath is made up of 70% lipid (Oulton and Mezei, 1976). Therefore, a burst of phospholipid and cholesterol synthesis is required for proper biogenesis and assembly of this elaborate membrane. We propose that apo A-I might be delivering lipid to the site of myelin formation. Our subcellular fractionation results support this hypothesis since one of the fractions, SO, which may represent transitional forms of the Schwann cell membrane involved in myelin assembly, was enriched in apo A-I associated with lipid. It is possible, however, that apo A-I association with this subcellular fraction arose during the fractionation of the tissue. The rapid rate of incorporation of cholesterol into myelin requires active transport and this role could also be assumed by apo A-I. The plasma HDL particles with which apo A-I normally associates contain an abundant amount of cholesterol esters. It has also been shown that cholesterol esters are very high in the avian sciatic nerve at the onset of myelination (Mezei et al., 1971) as well as during Wallerian degeneration (Mezei, 1970). The increase of cholesterol esterase parallels this process and it is proposed that the cholesterol esters are kept locally in the nerve until cholesterol is required to form the myelin membranes. It is plausible to think that apo A-I might associate with lipid particles containing a high amount of cholesterol ester and deliver these to their required site. The transport of these lipoprotein particles through the myelin could occur via the Schmidt–Lantermann clefts, which were once proposed to facilitate the transfer of components through the myelin sheath (Singer and Bryant, 1969).

The transfer of lipids to the myelin sheath has also been observed from the axon (Droz et al., 1978). Labeled lipids from the axon were observed to appear rapidly in the inner myelin sheath as well as at the outer Schwann cell cytoplasm. This transfer of lipids from neural to glial cell is proposed to be a mechanism for the maintenance of the glial cell myelin sheath. The transfer of lipids has been proposed to occur by phospholipid transport proteins (Droz et al., 1978). Another possibility is that the exchange or transfer of lipids is mediated by apo A-I–containing lipoproteins.

The uptake of cholesterol into the sciatic nerve from the blood has also been observed (Rawlins, 1973). One can visualize the delivery of cholesterol from circulating plasma lipoprotein particles to apo A-I–containing lipoproteins of the sciatic nerve.

One intriguing possibility is that active transcription and translation of the apo A-I gene in the developing avian nerve reflect a role unrelated to inter- or intracellular lipid transport, but indicate a possible involvement in the differentiation of Schwann cells. Our demonstration of the secretion of apo A-I by the sciatic nerve and the possible presence of this

antigen in the extracellular matrix supports this role. We speculate (following the suggestion of Mahley, 1988) that apo A-I in the chick sciatic nerve may interact with molecules in the extracellular matrix and may participate or modify cell-matrix or growth factor-matrix interactions.

The results of our investigations also raise a number of questions about the exact nature of the lipoprotein particles, the possible role of lipoprotein receptors, the identity of the cell types that synthesize and secrete apo A-I, and the regulation of the expression of the apo A-I gene in the chick sciatic nerve. Further lipoprotein fractionation and characterization, quantitative metabolic experiments with organ or cell culture, immunocytochemical electronmicroscopy, and in situ hybridization techniques should provide answers to these and other questions. Currently these experiments are actively being pursued in our laboratories.

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