SLOWLY MIGRATING AXONAL POLYPEPTIDES

Inequalities in their Rate and Amount of Transport between Two Branches of Bifurcating Axons

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ABSTRACT

Polypeptides in the dorsal root ganglion (L_5) of the adult rat were radioactively labeled, and components slowly migrating in the sciatic nerve (peripheral axons) and dorsal root (central axons) were analyzed, using SDS-polyacrylamide slab gel electrophoresis and fluorography. In particular, the transport rates and amounts of six major polypeptides, i.e., the triplet (reference 15; with mol wts of 200,000, 160,000, and 68,000 daltons), α- and β-tubulins and actin were compared between the two axon branches.

In peripheral axons, fronts of the triplet, tubulins, and actin migrate at 2–3 mm/d, 9–13 mm/d and ~19 mm/d, respectively. The corresponding values in central axons are 1–2 mm/d, 3–4 mm/d, and ~4 mm/d, indicating an obvious asymmetry in the transport rate between the two branches of bifurcating axons.

A greater amount of labeled triplet, tubulins, and actin each is found to migrate in peripheral than in central axons. Another striking aspect of asymmetry between the two branches relates to the tubulins/triplet ratio which is significantly higher in the peripheral branch.

Considerable proportions of radioactivities associated with tubulins and actin in the ganglion are nonmigratory, which are thought to derive mostly from periaxonal satellite cells. In contrast, most if not all of the labeled triplet is migratory, suggesting a virtual absence of triplet polypeptides in satellite cells.

The possible significance of peripheral-central inequalities in slow axoplasmic transport is discussed from the viewpoints of axon volume and axonal outgrowth.

KEY WORDS bifurcating axons · asymmetric transport · axonal outgrowth · tubulins and actin · 10-nm filaments · periaxonal satellite cells

Biological asymmetry in two branches of bifurcating axons that arise from the dorsal root ganglion cells has been shown under several circumstances. It is generally accepted that dorsal root ganglion cells show profuse chromatolytic changes after section of their peripheral axons but little if any reaction after section of their central axons (reference 6; for review, see reference 7). Changes in electrophysiological properties of the dorsal root ganglion cell body as well as a reduction in the axonal conduction velocity occur within 3 wk after

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the peripheral axotomy, whereas these changes are absent after central axotomy (8).

We have previously shown that the average rate of slowly migrating labeled proteins differs between the two axon branches, being higher in peripheral axons (17, 18). Two possibilities exist which can explain this asymmetry. First, each of the major polypeptide groups conveyed in the slow transport, shown to be the triplet (tentatively identified as neurofilaments), and α- and β-tubulins (15, 20), as well as actin (20, 26), may migrate more rapidly in peripheral than in central axons. Second, peripheral axons may be relatively more enriched with tubulins and actin which proved to migrate at faster rates than the triplet (15, 20, 26). The first possibility was supported by our previous fluorographic observations (26), and approximate flow rates of respective polypeptides in peripheral and central axons were estimated in the present experiment. Also, an attempt to quantitate the labeled tubulins and triplet in the present study indicates that the tubulins/triplet ratio is in fact higher in peripheral than in central axons. Further, fluorographic analyses of the dorsal root ganglion for eight successive weeks' postlabeling indicate that, unlike the radioactivity associated with the tubulins and actin, almost all the radioactivity associated with the triplet is migratory. This finding seems to suggest that 10-nm filaments in neurons (neurofilaments) and those in satellite cells are chemically dissimilar, though they are very similar in their electron microscope appearances.

MATERIALS AND METHODS

Treatment of the Animal and Nerve

Adult male albino rats (Wistar strain) weighing 250–300 g were used. Under ether anaesthesia, dorsal root ganglion (DRG;[1] L_5) was exposed by a partial laminectomy and L-[^{35}S]methionine (200 μCi in 0.4 μl) was injected via a glass capillary (20–40 μm in tip width) into the ganglion in two divided doses during the period of 10 min. At day 4 and at weeks 1, 2, 3, 4, 5, 6, and 8 after the injection, animals were killed by decapitation, and the sciatic nerve (with tibial, peroneal and sural branches reaching the ankle; 90–100 mm), L_5 ganglion, dorsal root (30–35 mm) and a portion of the spinal cord (hemi-cord) were dissected out. The tissue was placed on an ice-cold plastic plate, cut into consecutive 6-mm segments, or into 3-mm segments when specifically indicated, and kept frozen at −80°C until treated as below, generally within 1 wk.

Each segment was first ground in an ultramicro glass-glass homogeniser (Radnoti Glass Technology, Inc., Arcadia, Calif.) and further in a teflon-glass homogeniser, in the total volume of 400 μl of the medium which consisted of sodium dodecyl sulphate (SDS; 2.3%[2]), β-mercaptoethanol (5 mM), glycerol (10%, vol/vol) and Tris-HCl (62.5 mM), pH 6.8. The homogenate was then centrifuged at 1,500 *g* for 20 min at 25°C which resulted in an almost clear supernate in the cases of the dorsal root and the ganglion. In the case of the sciatic nerve, however, the supernate was overlaid by a thin, well-defined lipid layer, and in addition a small white pellet was produced. The clear portion was saved and subjected to further analyses.

SDS-Polyacrylamide Slab-Gel Electrophoresis

The nerve extract was incubated at 37°C for 15–20 min and then heated in a boiling water bath for 5 min, and an aliquot (generally 30 μl) was subjected to gel electrophoresis, essentially as described by Laemmli (19). The final composition of the separation gel was 10% acrylamide, 0.14% N,N'-bis-methylene acrylamide (Bis), 0.1% SDS, and 375 mM Tris-HCl, pH 8.8. The gel (10.5 × 13.5 cm; 2 mm thick) was prepared from a concentrated stock solution, and polymerized by the addition of N,N,N',N'-tetramethylethylenediamine (TEMED; 0.12%, vol/vol), and ammonium persulphate (0.048%). Stacking gel (5% acrylamide, 0.13% Bis, 0.1% SDS, and 125 mM Tris-HCl, pH 6.8; 1.3-cm long) was polymerized 12 h later by the addition of TEMED (0.2%, vol/vol) and ammonium persulphate (0.033%). The electrode buffer (pH 8.3) contained 0.1% SDS, 192 mM glycine, and 25 mM Tris. After applying the nerve extract and/or the mixture of molecular weight markers to respective slots (3- or 10-mm wide; 20 or 8 in number), electrophoresis was carried out at 12 mA/slab to achieve stacking (generally within 2–3 h) and then at 26 mA/slab until the tracking dye (bromophenol blue) reached the bottom (generally within 3–4 h). The gel was then stained by Coomassie Brilliant Blue R-250 (CBB; 0.25%), dissolved in 10% (vol/vol) acetic acid and 45% (vol/vol) methanol, destained, immersed in 3.7% (vol/vol) glycerol for 30 min (useful to prevent from later bending), and dried on a filter paper (Whatman 3 MM, Whatman, Inc., Clifton, N. J.) under a partial vacuum. Molecular weight markers are detailed in the legend to Fig. 1.

Fluorography

After electrophoresis, the gel was immersed in 10% (vol/vol) acetic acid for 20–30 min, and then processed for fluorography, essentially as described by Bonner and

[1] Abbreviations used in this paper: BSA, bovine serum albumin; CBB, Coomassie Brilliant Blue R-250; DRG, dorsal root ganglion; and, SDS, sodium dodecyl sulfate.

[2] Unless otherwise specified, percent concentration is given in wt/vol.

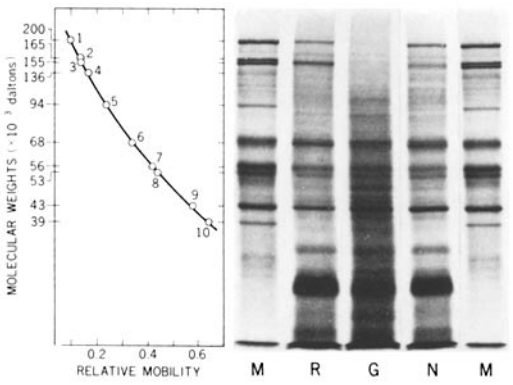


FIGURE 1 Polypeptides in the dorsal root, dorsal root ganglion, and sciatic nerve of the adult rat as revealed by SDS-polyacrylamide slab gel electrophoresis with subsequent CBB stain. Dorsal root, ganglion (L_5), and sciatic nerve were removed, washed repeatedly with ice-cold saline containing 5 mM β-mercaptoethanol, and finally with 5 mM β-mercaptoethanol in water. The tissue was then homogenized in a buffered medium, processed, and electrophoresed as described in Materials and Methods. Columns designated as M, R, G, and N are for marker proteins, dorsal root, ganglion and sciatic nerve, respectively. Approx. 120 μg of endogenous protein, as determined by the method of Lowry et al. (23), were applied to each slot (10 mm wide). A mixture of marker proteins listed below was electrophoresed in duplicate on each side of the gel, and their relative mobilities are plotted as a function of the logarithm of respective molecular weights given on the ordinate. [1] Rabbit skeletal muscle myosin (heavy chain), 2 μg. [2, 3, and 10] β'-, β- and α-Subunits, RNA polymerase from *Escherichia coli* (5), 3 μg. [4 and 6] BSA, dimer and monomer, 2 μg. [5] Phosphorylase *a* from the rabbit skeletal muscle, 1 μg. [7 and 8] α- and β-Tubulins from porcine brain (9), 7 μg. [9] Rabbit skeletal muscle actin, 5 μg. Literature references are for molecular weights.

Laskey (4). Finally, the dried gel was exposed to RP Royal X-Omat X-ray film (Eastman Kodak Co., Rochester, N. Y.) with or without brief flashing (21), and with a Cronex intensifying screen (Lightning-Plus; DuPont Instruments, Wilmington, Del.).

Electron Microscopy

Immediately after its exposure, the L_5 ganglion was fixed in situ with 2.5% (vol/vol) glutaraldehyde and 2% (vol/vol) formaldehyde in 100 mM sodium cacodylate buffer, pH 7.3. The ganglion was then dissected out, further fixed overnight at room temperature, washed with 10% sucrose dissolved in the same buffer, postfixed with OsO_4 in the same buffer, dehydrated with several changes of ethanol of gradient concentrations, and embedded in Epon 812. Thin sections (~800Å) were cut with a diamond knife, and examined in a Hitachi H-12 electron microscope.

Chemicals

L-[^{35}S]Methionine (820–1,245 Ci/mmol; 3 mCi/ml) was obtained from Radiochemical Centre, Amersham, U. K. The label was taken to dryness under a reduced pressure, and brought to a final concentration of 500 mCi/ml by the addition of distilled water. All the other reagents were of analytical grade.

RESULTS

Major and Minor Polypeptides Conveyed by Slow Transport

Among the migrating polypeptides visualized by fluorography, only six are major (Fig. 2*a-f*), in agreement with previous observations (15, 20, 26). These include a 43,000-dalton polypeptide with electrophoretic mobility identical with that of α-actin purified from rabbit skeletal muscle and will be referred to as actin below (our preliminary analyses with two-dimensional electrophoresis indicate that this polypeptide shows a pI slightly different from that of α-actin); 56,000- and 53,000-dalton polypeptides with electrophoretic mobilities corresponding to those of α- and β-tubulins purified from the porcine brain by two cycles of temperature-dependent assembly and disassembly with a subsequent gel filtration (13); and 68,000-, 160,000-, and 200,000-dalton polypeptides which were collectively referred to as the slow component triplet (15). The respective molecular weights of these polypeptides were determined on the basis of Rf values (Fig. 1), and also by the internal marker method described later (Fig. 4).

Most if not all of the radioactivity associated with the triplet leaves the ganglion within 2–3 wk of postlabeling (Fig. 2*c* and *d*). In contrast, considerable portions of the radioactivities associated with either tubulins or actin remain in the ganglion 5 wk (Fig. 2*f*) and even 8 wk after labeling the ganglion (fluorograph not shown).

A polypeptide with an apparent molecular weight of 170,000 daltons is seen to be confined to the ganglion, and to the peripheral and central segments adjacent to the ganglion (Fig. 2*a-g*). This may be either a nonmigratory neuronal polypeptide or one of nonneuronal origin. This polypeptide can be seen even at week 8 postlabeling (data not shown); this long lifetime makes the latter possibility more likely (see Discussion).

In addition to the six major migrating polypep-

tides mentioned above, fluorography reveals the occurrence of several minor components of various molecular weights. At day 4 and at weeks 1 and 2 postlabeling, polypeptides with mol wt of ≃35,000 and ≃25,000 daltons (marked with single and double white lines in the ganglion column, Fig. 2*a*, respectively) are seen to migrate at a rate similar to or a little less than that for actin (Fig. 2*a-c*). In addition, several minor polypeptides within the mol wt range from ≃220,000 to ≃60,000 daltons are seen to migrate at the rate similar to that for either actin or tubulins (Fig. 2*a-c*).

Flow Rates of the Six Major Polypeptides

A glance at the fluorographs in Fig. 2 shows that each of the triplet, tubulins, and actin migrates more rapidly in peripheral than central axons. On the basis of the distance between the migrating front and the ganglion at various time-points postlabeling (Fig. 2*a-c*), the front of the labeled triplet is estimated to migrate at 2–3 mm/d in the peripheral branch, and 1–2 mm/d in the central branch. The front of the labeled tubulins is estimated to migrate at 9–13 mm/d and 3–4 mm/d in the peripheral and central branches, respectively, on the basis of Fig. 2*a*, *b*, and *g*. The front of the labeled actin can be seen only at day 4 postlabeling (Fig. 2*a*), from which the front is estimated to migrate at ~19 mm/d and ~4 mm/d in the peripheral and central branches, respectively. The peripheral-central difference in migrating rates is evident with respect not only to the radioactivity front but also to the radioactivity peak (Fig. 3).

After the majority of radioactivity has passed distally, a weak but consistent radioactivity is detected in the proximal part of the axons. This is particularly impressive in case of actin, where the trailing radioactivity is seen almost evenly distributed over a wide range of axons at weeks 3, 4, and 5 postlabeling (Fig. 2*d-f*). The trailing of radioactivity is less apparent for tubulins and the triplet.

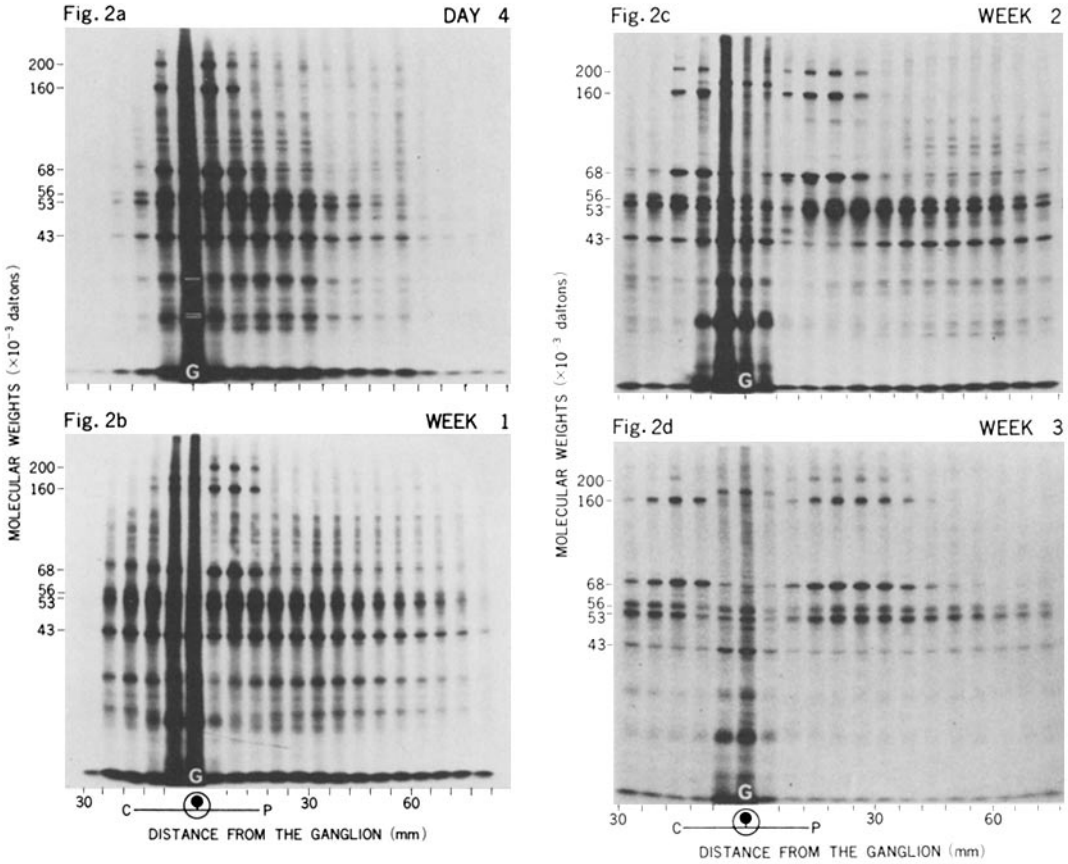
Internal Marker Method to Locate the Labeled Major Polypeptides in Coomassie Brilliant Blue-Stained Slab Gel

The major polypeptides are not only predominantly labeled but also heavily stained by CBB (Fig. 1; also, reference 15), indicating that they may well be located in CBB-stained gels. In practice, however, unless the nerve is washed repeatedly and the gel is destained thoroughly, the concomitant occurrence of a number of minor stained bands occasionally causes uncertainty about a precise location of each major polypeptide.

To assure an easier discrimination of the six major polypeptides, we added marker proteins to the nerve extract to be analyzed (Fig. 4). Proteins of known molecular weights were mixed with the nerve sample, the mixture was electrophoresed, and the gel was stained with CBB and destained. Half of each stained band, i.e., myosin, RNA polymerase (β'- and β-subunits), BSA monomer, α- and β-tubulins, and α-actin, was cut off with a fine blade. The gel was then processed for and subjected to fluorography, during which period the CBB stain was mostly decoloured. Fig. 4 demonstrates that the smallest major polypeptide migrates the same way as authentic α-actin, and doublet polypeptides the same way as α- and β-tubulins. The smallest polypeptide in the triplet migrates almost the same way as (but occasionally only slightly behind) the authentic BSA monomer; on the basis of this finding, the molecular weight given previously as 80,000 (26) should be corrected to 68,000. The second polypeptide in the triplet has an electrophoretic mobility in between those of β- and β'-subunits of RNA polymerase; its molecular weight is therefore estimated to be 160,000 daltons. The largest polypeptide in the triplet migrates only slightly behind but almost the same way as authentic myosin (heavy chain). Thus, the experiment shown in Fig. 4 demonstrates that the above-stated authentic proteins can be used effectively as markers to locate the labeled six polypeptides, even when the amount of six endogenous polypeptides in a given sample is too small to permit their clear discrimination on a CBB-stained slab gel.

Flow Amounts of the Six Major Polypeptides

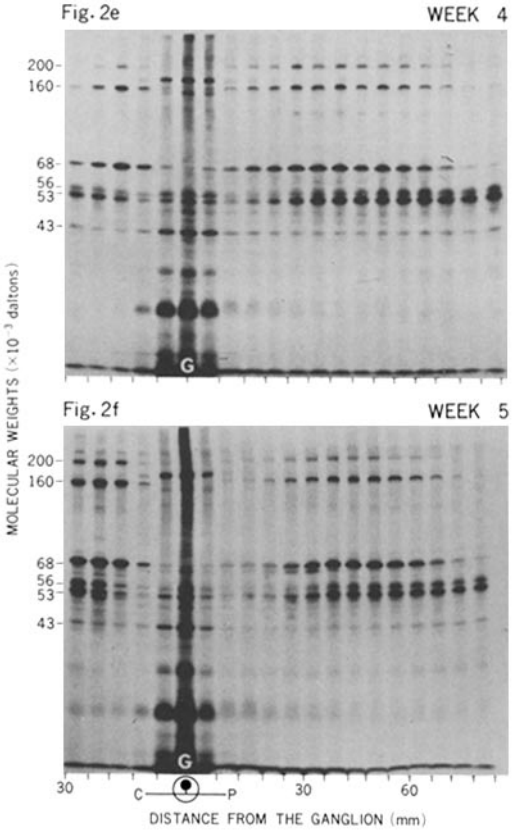
To know the respective amounts of the six major polypeptides, we attempted to determine the radioactivities of the triplet, tubulins, and actin separately. The problem was to find a time-point suitable for such a determination. In the case of actin, the migrating front is seen in the fluorograph only at day 4 postlabeling (Fig. 2*a*), and the radioactivity trails even at week 5 (Fig. 2*f*), which makes it hopeless to determine the amount of labeled actin. In the case of the triplet, the estimation is not difficult in that both the front and tail of the migrating radioactivity are within the stretch of axons when analyzed at weeks 3 and 4 postlabeling (Fig. 2*d* and *e*). At these time points, however, the front of labeled tubulins was beyond



the stretch of axons that was analyzed. The front of tubulins can be seen at day 4 (Fig. 2*a*) and at week 1 (Fig. 2*b*), but some portions of the migratory tubulins appear to remain in the ganglion at these time-points.

To find a point of compromise, we examined the front and tail of labeled triplet and tubulins more in detail, by analyzing the root and nerve taken at day 9 postlabeling and cut at 3-mm intervals (Fig. 2*g*). In the central branch, the labeled tubulins decline rather rapidly in segment C_{11}; in C_2, the extent of the blackening due to labeled tubulins appears to be slightly weaker than that in C_1 (Fig. 2*g*), indicating that a great majority of the migratory portion of labeled tubulins has already left the ganglion. In the peripheral branch, essentially all the labeled tubulins are recovered in segments from P_2 to P_{27} (Fig. 2*g*). In practice, the radioactivity of the tubulins was determined in the axon stretch corresponding to segments from P_1 to P_{28} in the peripheral branch, and from C_1 to C_{11} in the central branch. The radioactivities in segments P_1 and C_1 were not included in the total count to avoid the contribution of radioactivities due to the nonmigratory portion of labeled tubulins. In the experiment shown in Fig. 2*g*, the tubulin front is calculated to migrate at 9 mm/d in the peripheral branch. In other experiments, such as that shown in Fig. 2*a*, however, the tubulin front is found to migrate at 13 mm/d, which implies that the front can reach segment P_{39} at day 9 in this case. This leaves the possibility that, in some individuals, the leading portion of the tubulin radioactivity is missed from the determination. However, the radioactivity in ten 3-mm segments behind the front (estimated in P_{24}–P_{33}) was found to be <2% of the total tubulin radioactivity in P_2–P_{33}.

In the case of the triplet, the radioactivities in P_1 and C_1 were included in the total count, because almost all the triplet radioactivities prove migratory as already mentioned (Fig. 2*d-f*). The peripheral/central ratio of the labeled triplet estimated at day 9 (1.9; Table I) is essentially the same



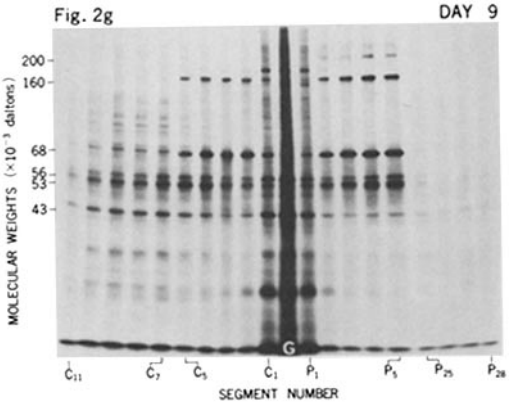


FIGURE 2 (*a–g*) Fluorographic analyses of polypeptides slowly migrating in the peripheral and central axons of the dorsal root ganglion cells of the adult rat. (*a–f*) The L_5 ganglion was labeled with L-[^{35}S]methionine and, at day 4 and at weeks 1, 2, 3, 4, and 5 postlabeling, the tissue was dissected out. Each of 6-mm consecutive nerve segments was homogenized in 400 μl of a buffered medium and, after an appropriate treatment, a 30-μl portion from respective segments was successively applied to each slot (3 mm wide), electrophoresed, and subjected to fluorography, as detailed in Materials and Methods. *P* stands for peripheral axons, *C* for central axons, and *G* for ganglion. (*g*) Nerve was dissected out at day 9 postlabeling, cut at 3-mm intervals, and thereafter treated the same way as in *a–f*. A 30-μl portion of the sample (200 μl) was applied to each slot. At the bottom is shown the segment number; e.g., P_1 denotes the first peripheral 3-mm segment, and C_1 the first central 3-mm segment. Four regions of consecutive segments, i.e., C_1–C_5, C_7–C_{11}, P_1–P_5, and P_{25}–P_{28} were electrophoresed.

as those estimated at weeks 3 and 4 (2.0 and 2.1, respectively), the time-points where the front and tail of the triplet radioactivity are more satisfactorily demarcated (Fig. 2 *d* and *e*).

In the experiments summarized in Table I, the location of the six major polypeptides was visualized in the CBB-stained gel by the use of authentic marker proteins (Fig. 4); appropriate gel portions were cut and the radioactivity of each endogenous polypeptide was determined in combined aliquots of samples from consecutive segments, the range of which is detailed above. It is shown that a significantly greater amount of labeled triplet and tubulins each migrates in peripheral than in central axons. Also, a greater amount of labeled actin, though at a rough estimate, is found in peripheral axons. Another notable aspect of the peripheral-central asymmetry relates to the tubulins/triplet ratio which is significantly higher in peripheral axons (Table I).

DISCUSSION

Our previous observation that the average velocity of slowly transported labeled proteins is higher in peripheral than in central axons of DRG cells of the adult rat (17, 18) appears to have two explanations. First, each of the three polypeptide groups (the triplet, tubulins, and actin) that comprise >80% of the slow transport (20) migrates at respectively higher rates in peripheral axons (Fig. 2; also, reference 26). Second, peripheral axons are relatively more enriched with tubulins and actin (Table I), which migrate at faster rates than the triplet.

The triplet polypeptides (212,000, 160,000, and 68,000 daltons) were initially suggested by Hoffman and Lasek to represent neurofilament subunits because of their prominence and identical distributions in the slow axoplasmic transport (15). This view is corroborated by recent demonstrations by Schlaepfer and his co-workers that poly-

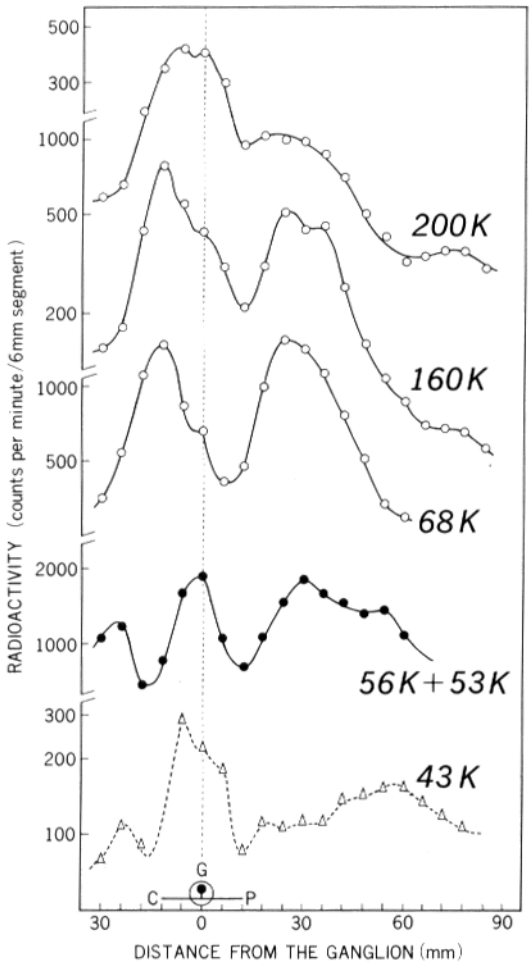


FIGURE 3 Flow profiles of the triplet polypeptides, tubulins, and actin in the peripheral and central axons of dorsal root ganglion neurons of the adult rat. The precise position of the triplet polypeptides (200,000, 160,000, and 68,000 daltons), tubulins (56,000 and 53,000 daltons), and actin (43,000 daltons) was determined by use of the internal marker method detailed in the text and in Fig. 4. Portions of the gel containing respective polypeptides were cut off, each of the gel blocks was incubated overnight at room temperature in 1.5 ml of Soluene-350 (Packard Instrument Co., Downers Grove, Ill.), further at 50°C for 5 h with shaking, and radioactivities were determined in a liquid scintillation spectrometer as previously described (1). For the triplet polypeptides and tubulins, the tissue was obtained at week 3, and for actin at week 2, postlabeling.

peptides with mol wts of 200,000, 150,000, and 69,000 daltons are the major components of intact neurofilaments isolated from the peripheral and central nervous systems (25, 30), and also that these polypeptides are selectively lost from the transected nerve during the same time interval in which neurofilaments are undergoing granular disintegration (31). Despite minor discrepancies in

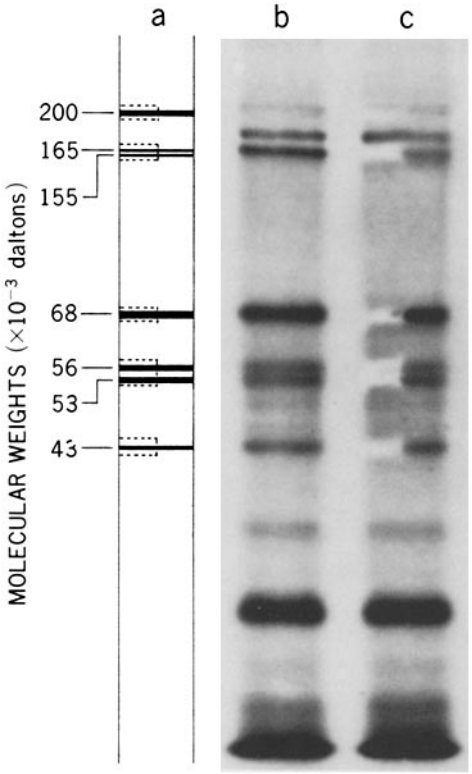


FIGURE 4 Internal marker method to locate the labeled polypeptides in CBB-stained gel. The labeled nerve extract obtained as described in Materials and Methods was mixed with appropriate quantities of marker proteins (cf. legend to Fig. 1). The mixture was dialyzed against the sample buffer, heated, electrophoresed, and stained by CBB. At the concentration used in this experiment (~15 μg of endogenous proteins), labeled polypeptides were almost unstained, visibly stained bands being attributable to exogenous marker proteins. Half of the stained band, each representing myosin (heavy chain), RNA polymerase (β′- and β-subunits), BSA (monomer), α- and β-tubulins, and α-actin, was carefully cut off with a fine blade, and the gel was then processed for and subjected to fluorography, during which period CBB stain was decoloured. (*a*) Schematic presentation of the CBB-stained gel, with cut-off portions enclosed with dotted lines. For the real photographic presentation of the stained gel, see Fig. 1. (*b* and *c*) Fluorograph of two adjacent columns in a single slab gel that was first stained by CBB and then subjected to fluorography. In *c*, half of the stained band was cut off as described above, before being subjected to fluorography.

apparent molecular weights, there is little room for doubt that we are dealing with the same set of polypeptides reported by these workers.

Unlike tubulins and actin of which some radioactivities remain inside the ganglion at week 5 (Fig. 2*f*) and even at week 8 postlabeling (fluorograph not shown), most if not all of the radioactivity associated with the triplet leaves the ganglion within 2–3 wk (Fig. 2*c* and *d*). The residual radioactivities for tubulins and actin may represent nonmigratory portions that remain in the neurone soma. However, it is more likely that the residual radioactivities are of nonneuronal origin, because autoradiographic study with [^{35}S]methionine and [^{3}H]glycine has indicated that neurons lose most of their radioactivity within a few hours' postlabeling, while other cell types, including oligodendrocytes and Schwann cells, retain their radioactivity for considerable periods of time (reference 16; a 170,000-dalton polypeptide in Fig. 2 is also likely to be of nonneuronal origin for this reason). It is shown in Fig. 5 that microtubules are prominent features occurring in periaxonal satellite cells (polar cells) in the rat DRG. Besides microtubules, a large number of 10-nm filaments are seen in satellite cells which are almost indistinguishable from those in axons (Fig. 5). Our present observation that most if not all of the labeled triplet is migratory seems to indicate that the triplet polypeptides (associated with 10-nm filaments in neurons) are absent in satellite cells. Though this strongly suggests a non-identity in molecular species of 10-nm filaments in neurons (neurofilaments) and 10-nm filaments in satellite cells, further analyses are needed to make that conclusion.

After the majority of actin radioactivity has passed distally, a weak radioactivity trails and distributes almost evenly over a wide range of axons, as seen at weeks 3, 4, and 5 postlabeling (Fig. 2*d–f*). It is improbable that this trailing portion represents a minor part of actin that is synthesized and loaded in the axoplasmic transport at later stages, because the chase of [^{35}S]-methionine by cold methionine 100-fold in amount and given 15 min later does not eliminate the trailing radioactivity. The possibility that the trailing radioactivity is due to a labeling of actin in extra-axonal structures (e.g., Schwann cells) is also negated by the observation that the nerve of the contralateral (unlabeled) side treated in the same way as the labeled nerve does not show any such radioactivity. It is most likely that a small portion of actin is deposited en route in its migration towards the periphery; this may be relevant to observations that actin-like protein is firmly attached to the axolemma (24) and that actin-like filaments (microfilaments) are anchored to the

TABLE I

Total Radioactivities Associated with the Triplet, Tubulins, and Actin in the Peripheral and Central Branches of the Dorsal Root Ganglion Cell Axons

	Mol wts	Radioactivities	
		Central axons	Peripheral axons
	×10^{-3} daltons	*cpm*	*cpm*
Triplet	200	790 ± 135‡	1,430 ± 196
	160	1,250 ± 130‡	2,190 ± 230
	68	3,050 ± 360*	6,240 ± 810
	Total	5,090 ± 580*	9,860 ± 1,160
Tubulins	56 53	4,840 ± 590*	15,700 ± 2,940
Actin	43	(3,300)	(6,900)
Triplet + Tubulins		9,930	25,560
Tubulins/Triplet		0.95 ± 0.06*	1.54 ± 0.14

The tissue was dissected out 9 d after labeling the ganglion, and the dorsal root and sciatic nerve were cut at 3-mm intervals. Each segment was homogenized in the total volume of 200 μl of the medium, and the supernate for electrophoresis was obtained as described in Materials and Methods. Aliquots of supernates from segments C_2–C_{11} were combined; aliquots were combined also from P_2–P_{28}. Samples C_2–C_{11} (in duplicate), C_1, ganglion, P_1 and P_2–P_{28} (in duplicate) were electrophoresed in a single slab gel (with 10-mm wide slots) with the addition of appropriate quantities of marker proteins (cf. Fig. 4) to each sample, and stained with CBB. Portions of the gel containing 200,000-, 160,000-, 68,000-, 56,000-plus 53,000-, and 43,000-dalton polypeptides were cut off, and the radioactivity in each gel was determined as described in the legend to Fig. 3. The radioactivity in the gel portion with no appreciable CBB-stain and with no blackening in the fluorogram (background radioactivity) ranged from 20 to 40 cpm per longitudinal 4 mm of the 10-mm column. Values for the triplet and tubulins are means ±SEM of five independent experiments. The difference between the peripheral and central axons is significant at $P < 0.02$ and at $P < 0.05$ for * and ‡, respectively (Student's t test). The counts for actin (the mean of five experiments) are only the rough estimate, because the front of migrating actin was beyond the stretch of axons analyzed (see text). The radioactivity that was missed from the determination would be greater in the peripheral axons, as the front of labeled actin migrates much faster in peripheral than in central axons.

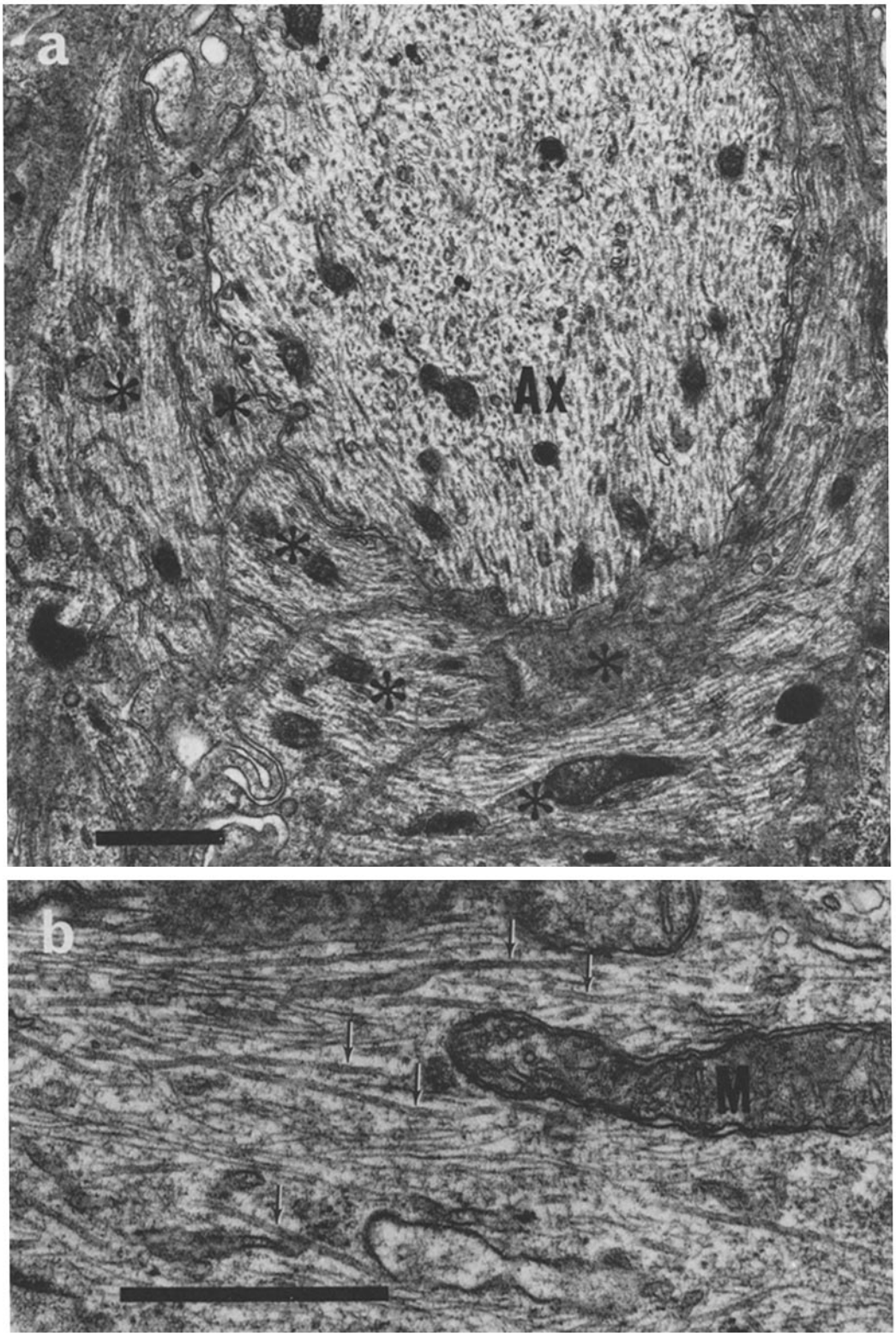


FIGURE 5 Electron micrographs of an axon and periaxonal satellite cells in the dorsal root ganglion (L_5) of the adult rat. (*a*) Cytoplasmic processes (asterisks) of the satellite cells are characterized by numerous microtubules, which run circumferentially around the axon (*Ax*). × 20,000. (*b*) Besides microtubules (arrows), many 10-nm filaments are seen in the satellite cell. *M*, mitochondrion. × 42,000. Bars, 1 μm.

plasma membrane (22).

The sum of the radioactivities associated with the triplet and tubulins in the peripheral branch is in great excess of that in the central branch (Table I). This observation is explained by the supposition that the total axon volume in the peripheral branch is greater than that in the central branch. The caliber of unmyelinated fibers, which comprise ~50% (2) or 60–70% (14, 28, 29) of the total number of axons, is reported six-fold thicker in the peripheral as compared with the central branch (12). The total length of the peripheral axons is supposed (though not measured) to be longer than that of the central axons. It would be relevant to refer to the electron microscope observation that the sum of the neurofilaments and microtubules correlates best with the amount of axoplasm (11).

Another notable quantitative asymmetry between two branches of bifurcating axons is that the tubulins/triplet ratio is significantly higher in the peripheral branch (Table I). There has been a considerable body of evidence to indicate that actin and tubulins are related to axonal outgrowth more directly than neurofilaments.The microfilaments are found in microspikes and in the periphery of the growth cone at the axonal tip (37); cytochalasin B that affects microfilaments (35) causes rounding up of growth cones, retraction of microspikes, and cessation of axon elongation (37). At the initial stage of the axonal outgrowth, microtubules are the prominent intraneuronal element, few if any neurofilaments being observed. Neurofilaments appear at later maturational stages and occur as the predominant component in mature neurons (references 2, 3, 27, 32, 36; Fig. 5). The importance of tubulins and actin in the axonal outgrowth is further supported by the observation that tubulins and actin are specifically increased in cultured sympathetic neurons when outgrowth is stimulated by nerve growth factor (10). Further recent studies of regenerating neurons indicate that slow component b (SCb; actin and the faster migrating portion of tubulins) is specifically increased in amount during axon regeneration, the flow rate, and amount of the triplet being unaffected (20). One may raise the speculation that a wealth of tubulins and actin relative to the triplet polypeptides in the peripheral branch (Table I) is indicative of its later maturation as compared with the central branch. It is at least possible that, because of its more voluminous entirety, the peripheral branch has to continue more active "perpetual growth" (33, 34) as compared with the central branch.

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