AXONAL TRANSPORT OF NEWLY SYNTHESIZED GLYCOPROTEINS IN A SINGLE IDENTIFIED NEURON OF *APLYSIA CALIFORNICA*

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ABSTRACT

Increasing amounts of glycoprotein synthesized from L-[^{3}H]fucose injected into the cell body of R2, an identified *Aplysia* neuron, were found in the right pleuro-abdominal connective. Autoradiography revealed that the glycoproteins were localized in the axon of R2. Glycoproteins appearing in the axon presumably were synthesized in the cell body, since no significant incorporation was observed when [^{3}H]fucose was injected directly into the axon. [^{3}H]glycoproteins were detected in the connective after a delay of 1 h after intrasomatic injection. Thereafter, transport from the cell body was rapid, and by 10 h after injection, 45% of the total neuronal [^{3}H]glycoprotein had appeared in the axon. By analysing the radioactivity in cell body and connective 4, 10, and 15 h after injection, we found that [^{3}H]glycoproteins were transported selectively compared to nonmacromolecular material. Sequential sectioning of the connective revealed that [^{3}H]glycoproteins were transported in discrete waves. The population of membrane-associated [^{3}H]glycoproteins in the axon differed from that in the cell body. Two of the five somatic components appeared to be transported preferentially. In addition a new component appeared in the axon 10 h after injection.

INTRODUCTION

Proteins (1), glycoproteins (2–4), mucopolysaccharides (5), and lipids (6), synthesized in the neuronal cell body, have been shown to be transported along the axon toward nerve terminals in a variety of animals. In all these studies, however, transport was examined in nerves composed of many thousands of fibers, with results which represent the average of events occurring in innumerable individual axons. In complex nervous tissue, transport processes within single neurons cannot be resolved and it is difficult to compare macromolecules remaining in the cell body with those selected for transport. This analysis is made possible by studying axonal transport in a single neuron.

Ambron et al. (7) showed that L-[^{3}H]fucose injected into the cell body of R2 was incorporated into a small number of membrane-associated glycoproteins. All of the glycoproteins synthesized are presumably neuronal, since fucose was found to be incorporated almost entirely within the nerve cell body. We have now examined the axonal transport of these newly synthesized glycoproteins in R2, the giant cell of the abdominal ganglion of *Aplysia*. Koike, Eisenstadt, and Schwartz (8) have

previously investigated the transport of acetylcholine formed from [³H]choline injected intrasomatically into R2. This neuron is anatomically advantageous for these studies because its major axon is large and courses unbranched within the right connective to the circumenteric ganglia, a distance of approximately 40 mm (Fig. 1, reference 9). We have found that the radioactive glycoproteins move rapidly along the axon, not in a single front, but rather in several distinct waves. In addition, the population of glycoproteins transported into the axon differs in some respects from that remaining in the cell body. These results suggest either that transport is selective or that glycoproteins, once in the axon, are metabolized differently from those which remain in the cell body.

MATERIALS AND METHODS

Intrasomatic Injection of R2

Aplysia californica weighing 60-120 g were supplied by Dr. R. C. Fay (Pacific Bio-Marine Supply Co., Venice, Calif.) and were kept at 15°C in circulating, well-aerated sea water. The intact central nervous system was removed from the animal and the cell body of R2 was injected with L-[³H]fucose (4.8 Ci/mM, New Eng-
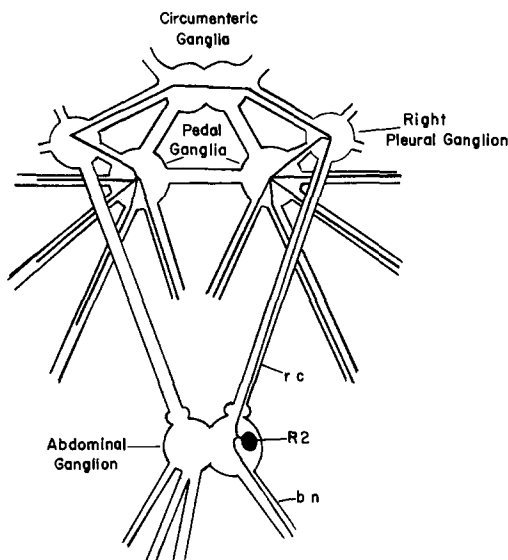

FIGURE 1 Diagram of the central nervous system of Aplysia showing the axon distribution of R2, after (9). The major axon courses in the right connective (rc) to the circumenteric ganglia, forming many branches first in the right pleuropedal ganglion and then in the left. These branches terminate in peripheral muscle. The minor axon exits from the ganglion through the branchial nerve (bn).

land Nuclear, Boston, Mass. [7, 9]). After injection, the nervous system was maintained at 15°C in a sea water supplemented with amino acids and vitamins (10). Intracellular recordings from R2 were made at the end of maintenance periods longer than 4 h. In no instance was there a significant change in the amplitude of the action potential or resting potential: action potentials were always greater than 65 mV and resting potentials greater than −45 mV (7). The general uptake of radioactivity which escaped into the bath after injection was estimated by counting radioactivity in the left connective. This nerve does not contain any of the processes of R2; usually it had approximately 0.1% of the radioactivity in R2 and never exceeded 0.5%.

Fractionation of the Cell Body and Axon

The washed nervous system was placed on the stage of a Mickle Gel Slicer (Brinkman Instruments, Inc., Westbury, N. Y.) and the right connective was carefully extended until coiling disappeared (8). Since the animal can elongate considerably, the nerve on the stage is not likely to be abnormally stretched; moreover, stretched molluscan axons are capable of propagating action potentials normally (11). The tissue was rapidly frozen with solid CO_2 and the location of the cell body of R2 was marked with ink. The frozen right connective was severed proximally just adjacent to the R2 cell body and distally at its entrance to the circumenteric ganglion. Usually the first cut was made just proximal to the bag cell region (7). Occasionally, however, the cell body of R2 occupied a more caudal position in the ganglion. When this occurred, the rostral part of the ganglion was taken with the connective. When the total glycoprotein content was to be determined the cell body and connective were homogenized separately and fractionated at 105,000 g as described in the accompanying paper (7). For studies of axonal transport, the frozen connective was sectioned sequentially into 1-mm segments. Each segment was homogenized at 4°C in a ground glass tissue grinder containing 200 μg carrier-nervous tissue in 0.05 M Tris-HCl (pH 7.6). Homogenates were then either treated with 10% TCA, 1.0% PTA[1] (each wt/vol) to precipitate total protein or were fractionated at 105,000 g to separate soluble and particulate glycoproteins (7). We checked the accuracy of sectioning by preparing a radioactive polyacrylamide gel uniformly labeled with [³H]fucose. The formed gel was frozen and sectioned in exactly the same manner as the connective. Radioactivity in each segment fluctuated around the mean with a coefficient of variation of 5.4% and a maximum range of ±15%.

Polyacrylamide Gel Electrophoresis

Radioactive glycoproteins were extracted from particulate cell fractions with SDS at 70°C. They were

[1] Abbreviations used in this paper: PTA, phosphotungstic acid; SDS, sodium dodecyl sulfate.

reduced and subjected to polyacrylamide gel electrophoresis in SDS (7) with dansylated bovine serum albumin as an internal standard (12). Samples were usually electrophoresed in duplicate. The duplicate gels were aligned using the standards and each was sectioned separately on the Mickle Gel Slicer. The amounts of radioactivity in aligned segments were summed, and the values plotted as a function of distance along the gel to yield a composite profile for each sample. Composite profiles were always similar to profiles plotted from individual gels and were constructed in order to minimize errors due to sampling or to incomplete solubilization of the gels.

The technique of intra-axonal injection (13) and the preparation of light microscope autoradiographs (14) have been published.

RESULTS

Appearance of [³H]Glycoprotein in the Right Connective

When L-[³H]fucose was injected into the cell body of R2 it was readily incorporated into a relatively small number of membrane glycoproteins (7). These newly synthesized glycoproteins did not remain in the cell body, however, but could be detected in the right connective after a delay of approximately 1 h (Fig. 2). The appearance of labeled glycoproteins was preceded by a small amount of soluble, nonmacromolecular radioactivity (Fig. 2). After the lag period, the proportion of total neuronal [³H]glycoproteins appearing in the connective increased at a rate of approximately 5% per hour until at least the tenth

hour. By this time 45% of the labeled glycoproteins had entered the connective (Fig. 2). At 15 h after injection, the proportion of total [³H]glycoprotein in the connectives had decreased to 30% (Fig. 2).

We found that labeled glycoproteins were preferentially transported relative to nonmacromolecular radioactivity, suggesting that the process was selective. At all times after the lag period, glycoproteins constituted a greater proportion of the total radioactivity in the right connective than in the cell body (Table I). Evidence for selective transport was also obtained by Koike et al. (9) who found that acetylcholine synthesized from [³H]choline injected into the cell body of R2 was enriched in the nerve with respect to other metabolites of choline. Despite the preferential efflux of glycoproteins, the proportion of total somatic radioactivity in glycoprotein was the same at 4 and 10 h (Table I), indicating that synthesis continues for at least 10 h after injection. With increasing distance from the cell body there was an enrichment of glycoproteins within the connective. This was determined by dividing the connective into three equal segments (proximal, intermediate, and distal) 15 h after injection. The more distal regions of the nerve contained increasingly greater proportions of glycoproteins which sedimented at 105,000 g (Table II).

An initial delay in transport of glycoproteins might be expected since the incorporation of labeled fucose into glycoprotein and the formation of cellular structures involves a complex series of reactions. We have shown, however, that mem-
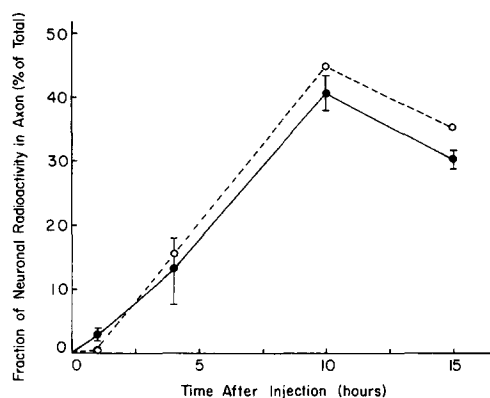

FIGURE 2 Transport of labeled glycoproteins into the right connective after intrasomatic injection of R2. The distribution between connective and cell body of [³H]glycoproteins (----) and of label in all chemical forms (-) was determined after fractionation (7) of the two parts of the injected neuron at 1 (n = 3), 4 (n = 8), 10 (n = 5), and 15 (n = 4) h.

TABLE I

Glycoprotein Content in Cell Body and Connective after Intrasomatic Injection of [³H]Fucose

Time after injection	Content of Glycoprotein	
	Cell body	Connective
(h)	(Percent of total radioactivity in glycoprotein)	
1	25 ± 4 (3)	<1
4	64 ± 2 (8)	77 (5)*
10	68 ± 4 (7)	81 ± 2 (5)
15	56 ± 1 (5)	70 ± 2 (4)

R2 was injected with between 5 and 43 pmol of [³H]fucose. At the times indicated, the abdominal ganglion and right connectives were homogenized and fractionated as described in the accompanying paper (7). Under our conditions of counting 2,200 cpm was equivalent to 1 pmol.

* Determination includes three connectives which were combined.

TABLE II

Enrichment of Particulate Glycoproteins along the Connective

	Distribution		
Total Radioactivity (cpm)	Prox-imal 8,350	Inter-mediate 5,940	Distal 574
	(Percent of total radioactivity)		
Total acid soluble radioac-tivity	57.8	13.7	0
Soluble glycoproteins	0.6	1.8	2.4
Particulate glycoproteins (SDS extract)	39.9	74.9	92.3
Residual radioactivity	1.7	9.6	5.2
Total macromolecular ra-dioactivity	42.2	86.3	100

15 h after intrasomatic injection of R2, the right connective, which was 27 mm long, was cut into three sections of equal length. Each section was fractionated at 105,000 g (7).

brane-bound, labeled glycoproteins are present in the cell body by 1 h (Table I). Since particulate glycoproteins were present in the cell body, but were not yet transported, it is likely that additional processing is required after fucosylation for these newly synthesized glycoproteins to enter the axon.

The proportion of particulate glycoproteins in cell bodies 1 h after injection was less than half that found at 4 and 10 h (Table I). The amount of GDP-fucose might limit synthesis at 1 h. We therefore analyzed the soluble fraction from cell bodies 1 and 4 h after injection; $10 \pm 2\%$ $(n = 3)^2$ of the total radioactivity was adsorbed by charcoal at 1 h, and the percentage at 4 h was not higher $(6.4 \pm 2 (n = 5))$.[3]

Localization of Labeled Glycoproteins to the Axon of R2

We were not able to show by biochemical analysis that the labeled glycoproteins which appeared in the connective were intra-axonal, because it was not possible to separate the axon of R2 from other structures in the connective. Since soluble radioactivity was found in the connective before the appearance of [3H]glycoproteins,

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[2] All values reported are ± standard error of the mean followed by the number of experiments in parentheses.
[3] Evidence that this material is GDP-fucose was obtained by electrophoretic and chromatographic analysis (7). It was not definitively identified, however.

[3H]fucose might have passed through the ax-olemma to be incorporated into glycoprotein in surrounding axons, glial cells, or connective tissue. In collaboration with Dr. E. B. Thompson in our laboratory we therefore examined the distribution of radioactivity by autoradiography in transverse sections of the connective 4, 10, and 15 h after injecting the cell body of R2. At each time period virtually all silver grains were found over the axon of R2, the largest process in the right connective (Fig. 3 and see Thompson et al. [14 and manu-script in preparation]). We chose to show an autoradiograph which was exposed for a long time in order to reveal, with a high degree of sensitivity, the presence of radioactivity which might be localized outside of the axon. The axon does not appear as a simple tube, but rather is stellate in cross section with glial cells surrounding and deeply invaginating the process (Fig. 3). This arrangement of glial cells has been more clearly shown by electron microscopy (14, 15). We found essentially background labeling over all glial cells, both those invaginated deeply within the structure of the axon, and those on the outer perimeter. Moreover, no label was observed in the other structures of the connective including the axons of other neurons and connective tissue (Fig. 3 and reference 14).

Absence of Fucose Incorporation into Glycoprotein within the Axon

We have shown that the radioactive glyco-proteins in the right connective are located only within the axon of R2, and we have presented evidence which indicates that incorporation of fucose into glycoprotein occurs almost entirely within the injected neuron. If it can be shown that fucosylation does not occur in the axon, then we can conclude that the appearance of labeled glyco-protein in the nerve resulted entirely from axonal transport. A suggestion that the axon cannot incorporate exogenous fucose into glycoproteins was obtained by incubating the connective (severed at both ends) in [3H]fucose for 4 h (7). Under these conditions, autoradiography revealed essentially background radioactivity over axoplasm while surrounding glia and connective tissues were heav-ily labeled (Fig. 4). Uptake of fucose into the axon might have been the limiting factor, however.

Direct evidence that fucosylation of glycopro-tein does not occur within the axon was previously noted by Treistman and Schwartz (13) who in-jected [3H]fucose directly into the axon of R2
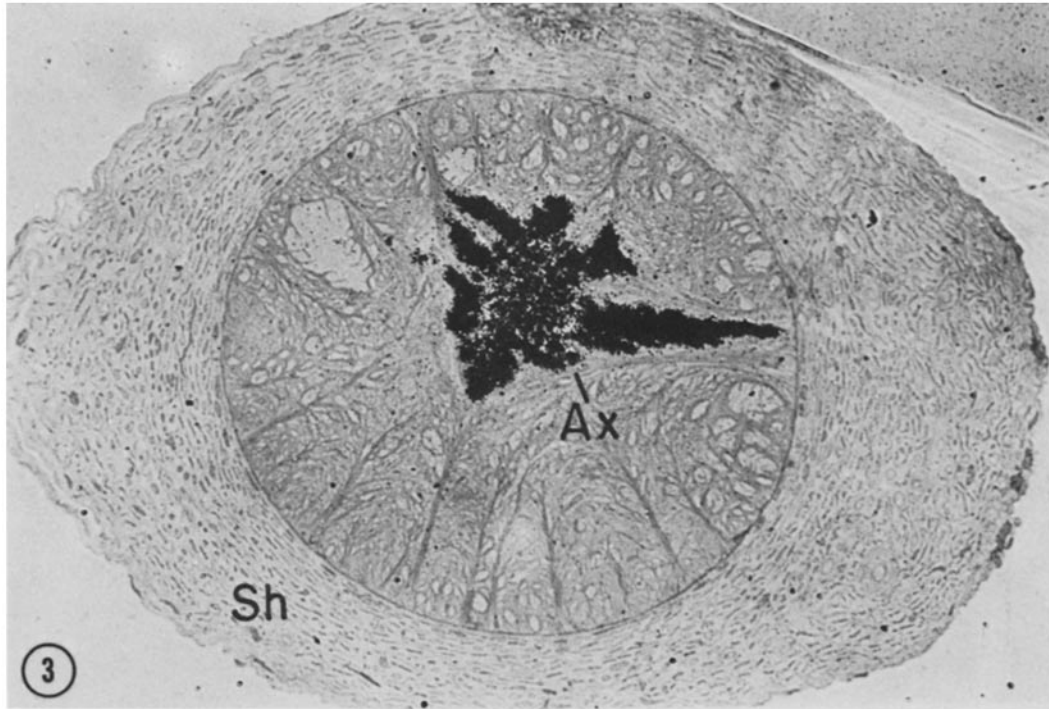
FIGURE 3 Light microscope autoradiograph of a cross section of the right connective 15 h after intrasomatic injection of R2. Silver grains are located over the axon of R2 (*Ax*); *Sh*, connective tissue sheath. × 415. (Prepared in collaboration with Dr. E. B. Thompson.)

within the right connective. They found that 4 h after injection, at which time 24-46 pmol of [³H]fucose were retained by the axon, only a negligible amount of radioactivity was incorporated into glycoproteins. In collaboration with Dr. S. N. Treistman in our laboratory, we extended their earlier study by injecting equivalent amounts of fucose, but waiting 24 h before measuring the glycoprotein content. The results were similar: only 1.0% of the retained radioactivity was incorporated, even though 5% of the total axonal radioactivity was in material tentatively identified as GDP-fucose[3], an amount which we found to be sufficient for maximal synthesis of labeled glycoproteins in the cell body.

The Distribution of Newly Synthesized Glycoprotein along the Axon

In order to investigate the rate of transport of labeled glycoproteins and their distribution along the axon, we cut right connectives between the abdominal and circumenteric ganglia into 1-mm sections at 4, 10, and 15 h after injection of the cell

body. By analyzing each section for its content of labeled glycoprotein by acid precipitation, we found that glycoproteins were concentrated at certain points along the axon (Fig. 5). Radioactivity at each of these points was discrete, often being confined to a 1-mm segment. The number of these concentrated peaks of radioactivity and their distance from the cell body depended on the length of time after injection. From the distances traveled, we estimated a rate of 50-70 mm per day for the radioactivity in the most distal peak. This rate is only approximate since we found considerable variation from animal to animal. Labeled glycoproteins were usually detected in the region of the circumenteric ganglia by 15 h (Fig. 5 B). The amount depended on the length of the connective. In order to determine if soluble and particulate glycoproteins had a similar distribution along the axon, we fractionated at 105,000 *g* each 1-mm segment of the connective 4 and 10 h after intrasomatic injection (Fig. 6). At both time periods radioactivity in both soluble and particulate glycoproteins was distributed in discrete peaks. Within each axon, however, the localization
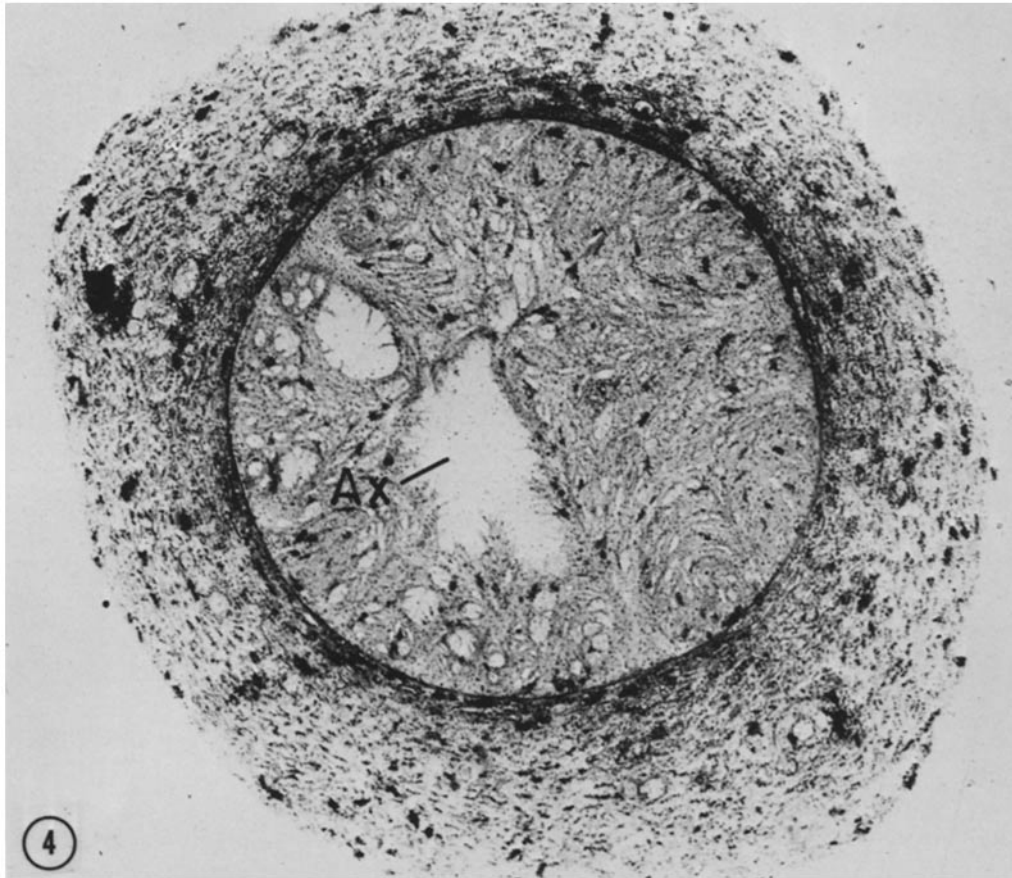
FIGURE 4   Light microscope autoradiograph of a cross section of the right connective after 4 h incubation in a supplemented sea water containing [³H]fucose. Silver grains are located primarily over components of the connective other than axons. *Ax*, axon of R2. × 345. (Prepared in collaboration with Dr. E. B. Thompson.)

of the particulate and soluble glycoproteins did not coincide, although the most distal peak in each had moved approximately the same distance (Fig. 6).

Electrophoretic Profiles of
[³H]Glycoproteins in the Cell
Body and Axon

Ambron et al. (7) found that [³H]fucose injected into the cell body of R2 was incorporated into five major membrane-associated glycoprotein components. Electrophoretic patterns of the labeled glycoproteins were similar at 4 and 10 h after injection. A characteristic of these profiles was that components I and V had the greatest proportion of the radioactivity (7). We have now extended these studies by examining the somatic glycoproteins 15 and 24 h after injection. At these longer times, component I remained prominent, but there was a marked reduction in radioactivity in components IV and V (Fig. 7 A, 8A). These changes from the 4- and 10-h patterns were observed in all six 15-h cells and three 24-h cells examined. A similar profile was seen when a 15-h cell body was extracted directly with hot SDS, indicating that artifactual proteolysis of components IV and V during fractionation in buffer was not responsible for the change.

The profiles of [³H]glycoproteins appearing in the right connective were different from those observed in the cell body. Patterns in the connective at 4, 10, 15, and 24 h after injection (Figs. 9 A, 9 B, 7 A, 8 A) showed a distribution of radioactivity essentially reciprocal to that in the cell body. At all times radioactivity in peak I was reduced in the connective and peaks IV and V were more promi-

nent. This suggests that components IV and V might have been preferentially transported in the axon, while component I remained in the cell body.

Another difference between the cell body and axon was the appearance of a new labeled glycoprotein in the connective. This high molecular weight component, which was never seen in the cell body, was occasionally observed after 10 h (Fig. 9 B) but was always seen in the axon at 15 and 24 h (arrow, Figs. 7 B, 8 B). Alteration in the profile of glycoproteins in the connective with time might result from turnover or processing.

DISCUSSION

Glycoproteins associated with particulate subcellular components are rapidly transported along axons toward nerve terminals (2–4). In *Aplysia* we found that the most rapid [³H]glycoproteins were transported at approximately 50 mm/day, a rate similar to that of fast transport in other poikilotherms (4, 5). Most of the fucose-labeled glycoproteins migrating in the axon of R2 were particulate, although soluble glycoproteins in amounts sufficient to measure reliably were also detected (Fig. 6). We do not know the physiological or biochemical function of these macromolecules, but

we presume that they serve to replenish various membrane systems of the axon and nerve terminal.

Characterization of [³H]glycoproteins in Axon and Cell Body

The population of labeled glycoproteins transported in the axon after intrasomatic injection of [³H]fucose differed from that remaining in the cell body (Fig. 7, 8). Not only was there an enrichment of particulate macromolecules in the axon compared to the cell body (Table I), but also component I, a major somatic glycoprotein, was greatly reduced in the axon (Figs. 7 B, 8 B, 9 A, 9 B). These differences between the glycoproteins in the two regions of the neuron suggest that transport is a selective process. Alternatively, glycoproteins might be processed differently in the axon and cell body. Proteins are known to vary greatly in their rates of turnover (16), and glycoproteins have been shown to turn over more rapidly in one cellular compartment than in others (17). In *Aplysia*, the average turnover of proteins was estimated at 0.1% per hour (18). If the quantitative differences in axonal and somatic glycoproteins are due to differential turnover in the two regions of the
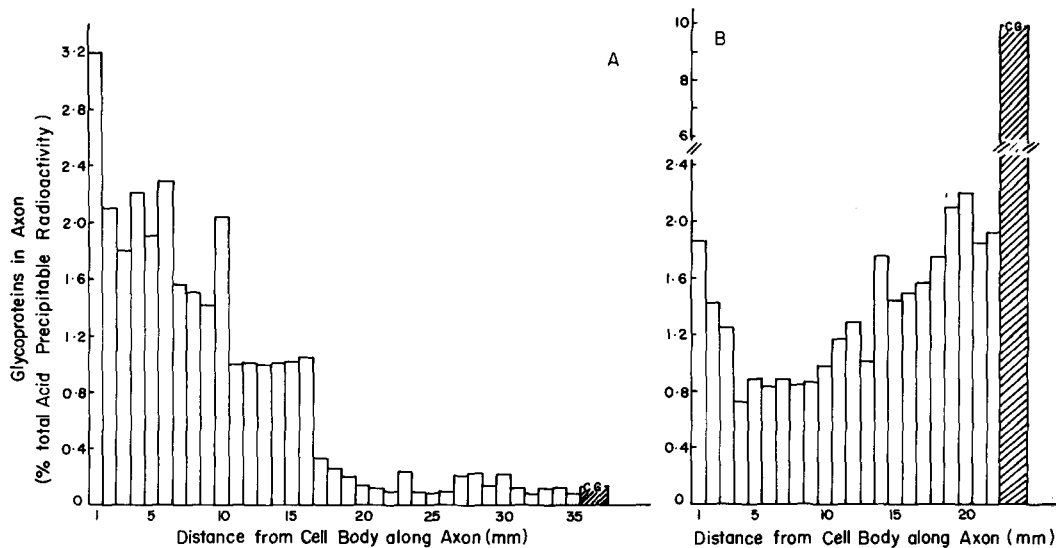

FIGURE 5 Distribution of [³H]glycoproteins along the axon of R2. Tissue was frozen and the connective severed from the abdominal ganglion. The connective was cut into 1-mm segments and each segment was homogenized in 10% TCA, 1% PTA. In order to determine total neuronal [³H]glycoproteins the abdominal ganglion, containing R2's cell body, and the circumenteric ganglia were also analyzed. (A) 10 h after injection. 1% of total is equivalent to 155 cpm. (B) 15 h after injection. 1% of total is equivalent to 320 cpm. This connective was shorter than the average which was approximately 30 mm. Bar marked *CG* is acid precipitable radioactivity in entire circumenteric ganglia.
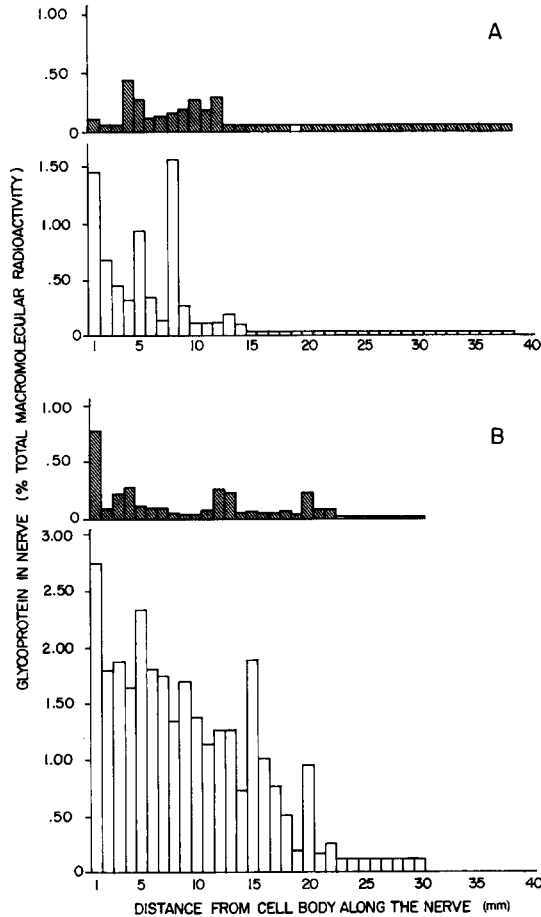
FIGURE 6 Distribution of particulate and soluble [³H]glycoproteins along the axon of R2. The entire length of the right connective from the abdominal ganglion to the right pleural ganglion was sectioned into 1-mm segments. Each segment was homogenized in buffer, fractionated at 105,000 g, and analyzed for its content of soluble (hatched bars) and particulate (open bars) [³H]glycoproteins. The abdominal ganglion containing the cell body of R2 was also analyzed. (A) 4 h after injection. (B) 10 h after injection.

(17). We found that a new high molecular weight component, which was never seen in the cell body, appeared in the axon 10 h after injection (Figs. 7 B, 8 B, 9 B). Since we have shown that [³H]fucose cannot be incorporated into macromolecules within the axon, the new component probably arises through a transformation of some other glycoprotein, possibly component I. Alternatively, since we have found that soluble glycoproteins were also rapidly transported along the axon (Fig. 6), they might also serve as precursors of the new particulate component. In accord with this possibility, we found that the proportion of soluble glycoproteins in the axon diminished with time after injection: after 10 h, 40 ± 8% (n = 4) of the total soluble glycoproteins were in the axon; by 15 h, this value was reduced to 14 ± 2% (n = 4).
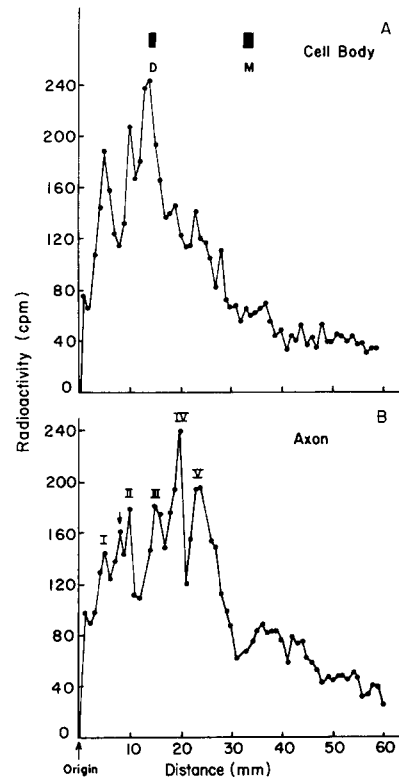

FIGURE 7 SDS polyacrylamide gel electrophoresis of labeled particulate glycoproteins in cell body and axon 15 h after intrasomatic injection of [³H]fucose. The cell body was removed from the ganglion. The isolated cell body and right connective were homogenized separately and fractionated at 105,000 g. The particulate fraction was extracted into 1% SDS, 1% 2-mercaptoethanol, 10 mM sodium phosphate (pH 7.2) and electrophoresed for 4 h at 6.5 mA/gel.
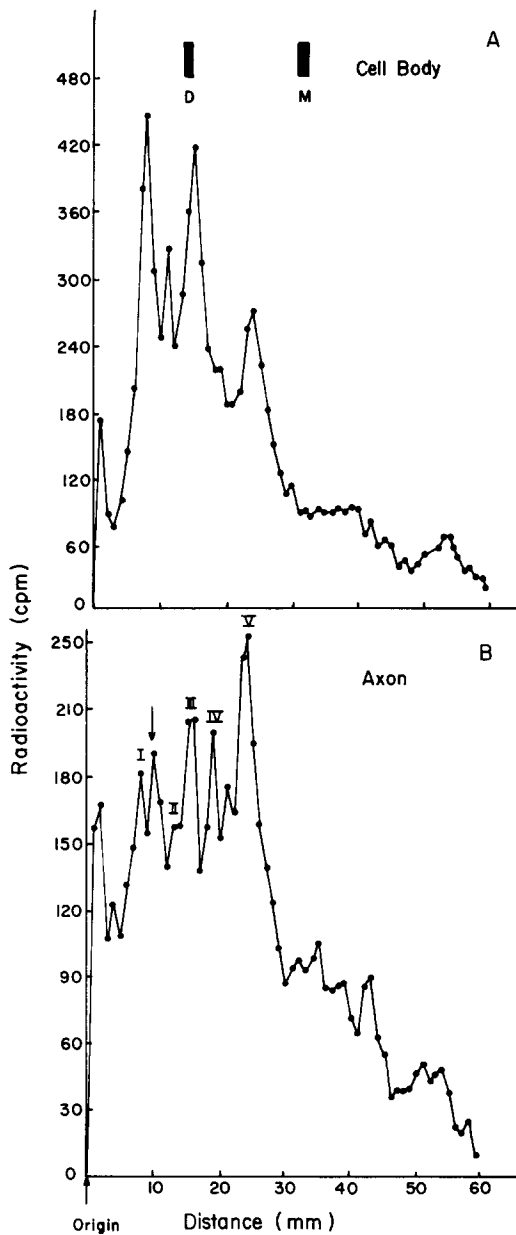
neuron, turnover must occur at a considerably more rapid rate than the average. A rapid turnover of a low molecular weight protein labeled with [³H]leucine has been reported by Gainer (19) and Wilson (20) in R15, another identified neuron of Aplysia.

It is also possible that certain glycoproteins are altered or processed as they are transported, or when they reach their final destinations. Evidence for the incorporation of glucosamine, but not fucose, into transported glycoproteins at nerve endings has been presented by Zatz and Barondes

FIGURE 8 SDS polyacrylamide gel electrophoresis of labeled particulate glycoproteins in cell body and axon 24 h after intrasomatic injection of [³H]fucose. The isolated cell body and right connective were homogenized separately and fractionated at 105,000 g.

Possible Mechanisms of Axonal Transport

Rates from 10 to 2800 mm/day have been recorded for translocation of material in axons (21). Although rates of axonal transport may be estimated by measuring appearance of radioactive materials at their final destination at nerve terminals (2–4), fine resolution of transport is best examined by sequential sectioning of the axon. This approach, which was introduced by Ochs and his collaborators in the sciatic nerve, revealed that protein labeled after intraganglionic injection of [³H]leucine moved along the nerve in a wave front (1). These transport kinetics undoubtedly result from the averaging of separate waves moving within many thousands of individual axons. The movement of material in any single fiber might vary considerably from the average, since individual neurons can be expected to vary in both function and metabolic activity.

We have found the mode of transport in an individual *Aplysia* neuron to be different from that in the sciatic nerve. Both membrane-associated and soluble glycoproteins proceeded along the axon of R2 in several discrete waves (Fig. 6). Similar distinct waves also could be detected in the nerve cord of the crayfish after intraganglionic injection of [³H]leucine (22). Although we cannot be certain, we assume that the difference between transport in invertebrates and in the sciatic nerve resulted primarily from the relative simplicity of the invertebrate preparations and not from any inherent differences in the mechanism of transport.

We suggest that there are three general explanations for the mode of axonal transport observed in R2. The least likely explanation would result from the periodic efflux of glycoproteins from the cell body, either because of pulsatile synthesis or periodic packaging. According to this hypothesis, the discrete waves seen in Figs. 5 A, 5 B, and 6 would all be moving at the same rate of about 50–70 mm/day. The distance between wave crests would represent periods of inactivity between bursts of transport. In accord with this first hypothesis, we have found that incorporation of [³H]fucose into glycoproteins has not ceased in the cell body by 10 h after injection. Continuing synthesis must occur in order to account for the constant proportion of somatic [³H]glycoprotein from the fourth to the tenth hour, the period when the maximal amount of glycoprotein leaves the cell body (Table I, Fig. 2). At present, we have no experimental evidence against pulsatile synthesis or packaging.

The second general hypothesis is that transport is in some way influenced by the geometry of the axon. The simplest model would involve variation in the cross-sectional area of the axon lumen along the length of the nerve. Even though material
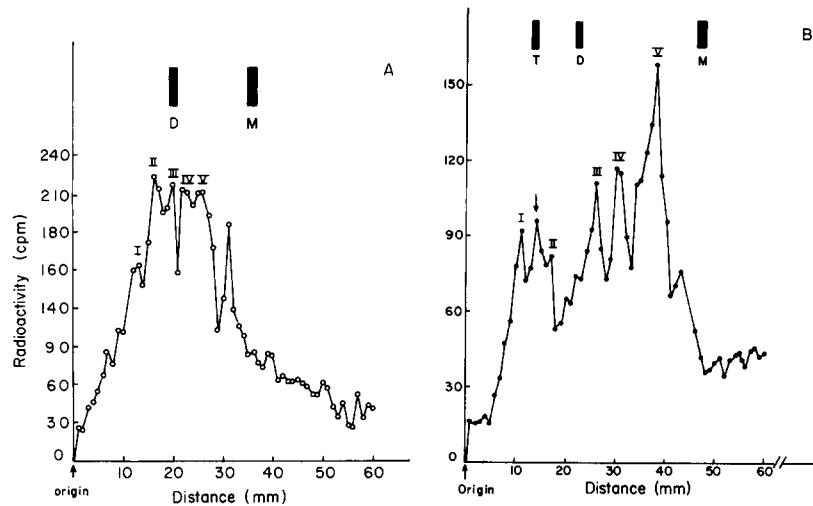
FIGURE 9 SDS polyacrylamide gel electrophoresis of labeled particulate glycoproteins in the axon after intrasomatic injection of [³H]fucose. (A) 4 h after injection. The right connectives from three injected cells were combined and fractionated. The particulate fraction was extracted with SDS and electrophoresed. (B) 10 h after injection. The particulate fraction from a connective, after extraction with SDS, was reduced and alkylated (7). The extract was dialyzed against water, lyophilized, and extracted into sample buffer. Electrophoresis was performed on a 10-cm gel at 7.0 mA for 5 h. Only the top two-thirds of the gel containing protein with molecular weight greater than about 50,000 daltons was sectioned.

moving from the cell body might contain a constant concentration of [³H]glycoproteins, radioactivity in sections of nerve equal in length might appear to vary considerably, since individual samples would actually contain variable amounts of the labeled axoplasm. Consistent with this hypothesis, Coggeshall (15) and Thompson et al. (14) have shown that the axon of R2 in the right connective is quite irregular in cross section (Also, see Fig. 3). In collaboration with Dr. E. Thompson we sectioned the right connective at 15 h after an intrasomatic injection of R2, using alternate 0.5-mm segments for autoradiography and for scintillation counting. The cross-sectional area of the axon was estimated by tracing its outline on paper (Fig. 3), cutting out the trace, and then weighing the paper. Although this determination was preliminary since we did not section the nerve completely, we found no indication that the distribution of radioactivity was correlated with cross-sectional area. Moreover, even though the lumen of the axon displayed a wide variety of configurations, we observed at most only a twofold variation in cross-sectional area.

It is therefore unlikely that local variation in the gross volume of the axon produces the waves of transported [³H]glycoproteins. Nevertheless, some geometrical factor other than cross-sectional area might account for the discrete mode of transport observed. Transport might occur in specific channels within the axon and the number of channels might not be directly related to the cross-sectional area. In accord with this idea are the observations of Thompson et al. (14) who found by light autoradiography that silver grains representing transported [³H]glycoproteins were unevenly distributed in cross sections of the axon of R2. The densest labeling occurred over areas just under the perimeter of the axon, close to the glial cell investments. Axoplasm in the central parts of the process was relatively unlabeled. It was also noticed in the electron microscope that various organelles were not distributed homogeneously within cross sections of the axon, but rather that they accumulated in dense clusters (14).

The third hypothesis to explain the discrete distribution in the axon is that various membranous organelles are transported at different rates, and that each of the observed waves carries newly synthesized glycoproteins characteristic of the membranes of a particular type of organelle. The presence of membranous organelles in the axon of R2 has already been described (14, 15) and radioactivity from [³H]fucose injected intrasomatically was seen primarily to label these membranous structures in both axon and cell body (14).

We have not yet been able to carry out definitive experiments to discriminate among these three general hypotheses. If transport is pulsatile or determined by local geometrical features of the axon, the distribution of glycoproteins in the various waves might be expected to be similar. If, on the other hand, each individual wave contains a special group of organelles, we might expect glycoproteins in the individual waves to vary. A comparison of electrophoretic profiles of [³H]glycoproteins in the individual waves within a single axon might help to distinguish between these hypotheses. Resolution is now hampered because the injected [³H]fucose continues to be incorporated within the cell body throughout the experiment. We are currently studying conditions which might stop the incorporation after short periods of time.

It should be noted that the three general hypotheses are not necessarily mutually exclusive. Thus it is possible that axonal transport of membranous organelles might tend to occur at characteristic velocities, but also be pulsatile and controlled by geometrical features of the axon. These mechanisms, which would be extremely difficult if not impossible to resolve in a population of nerve cells, can now be approached in a single neuron.

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