Transport of Protein by Goldfish Optic Nerve Fibers

Abstract. *After tritiated leucine was injected into the eye of goldfish, radioactive protein synthesized by the ganglion cell bodies moved down the optic axons at an average rate of 0.4 mm per day. Radioautograms of the optic tectum in which these axons end show that, as early as 24 hours after the injection, before the radioactivity in the tectal layer containing the optic axons had risen above background level, the layer containing the axon terminals was already heavily labeled. The radioactivity in the terminals reached a maximum about 48 hours after the injection and remained approximately constant for at least 23 days thereafter, whereas the radioactivity in the fiber layer increased significantly during the same interval, as the slowly moving protein component entered it. Thus there appears to be a special mechanism for rapid transport of protein from the cell body to the synaptic terminals, as well as a slower movement of protein down the axon.*

The idea that the processes of nerve cells are dependent on the cell body for their maintenance and integrity is as old as the observation that an axon severed from the cell body degenerates (*1*). The observations of Weiss and Hiscoe (*2*) on the damming of axoplasm in constricted mature nerve fibers as well as regenerating fibers gave a more coherent shape to this idea, emphasizing the fact that materials from the cell body were constantly moving down the axon. With the application of radioactive tracers to this problem, work on axonal transport has been rapidly accelerating (*3–7*). Protein transport has received particular attention, because the principal site of protein synthesis is the neuronal cell body.

In the present experiments the rate of transport of protein along goldfish optic axons was determined by measuring the distribution of radioactivity along the optic nerve at various times after the injection of tritiated leucine into the eye. The radioactive amino acid enters the ganglion cell bodies, where it is incorporated into protein which is then transported down the optic nerve fibers. My results indicate that, in addition to a slow transport (a fraction of a millimeter per day) of protein within the axon, there is a separate protein component which is rapidly transferred (several millimeters per day) from the cell body to the nerve endings, without becoming dispersed in the axonal protein compartment.

Fish (6 to 10 cm in body length) were injected by the technique of Taylor and Weiss (*5*) with 1 to 3 μl per eye of leucine-4,5-^{3}H solution (2 μc/μl). They were kept at 21° ± 1°C. After an appropriate interval, the eyes, optic nerves, and tecta were removed, fixed in Bouin's solution for 48 to 72 hours, then extracted with fat solvents so that only the radioactivity incorporated into protein was retained in the tissue (*8*). In the 3- to 5-mm length of optic nerve between the eye and optic chiasma, the radioactivity in successive 0.25-mm segments was measured by a scintillation-counting technique (*9*). The same scintillation method was used in some experiments to assay 1-μl samples of vitreous humor aspirated from the eyes before they were fixed. Radioautograms of the retinas and brains were prepared according to the method of Kopriwa and Leblond (*10*).

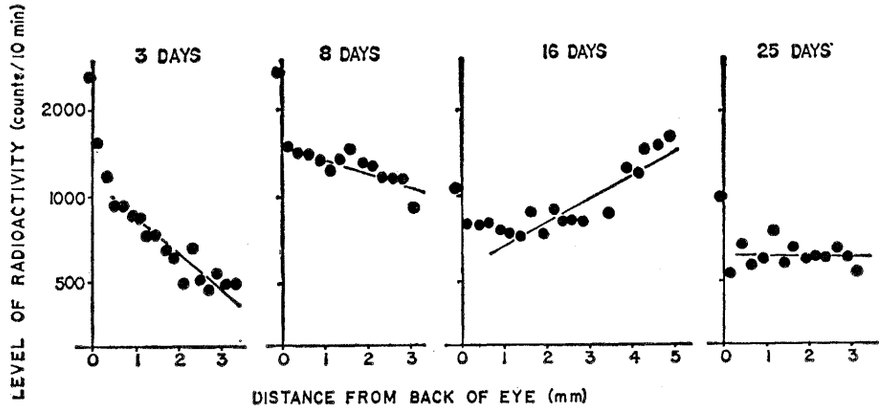

Fig. 1. Some typical examples of the distribution of radioactive protein in optic nerves at various times after the injection of leucine-^{3}H into the eye. Zero distance on each nerve is taken as the point at which the choroidal pigment is no longer visible.

The first evidence that protein was being transported along the nerve was the fact that the radioactivity in the nerve as a whole increased for several days after the injection, although samples of the vitreous humor and radioautograms of the retina showed that after 24 hours there was no longer any appreciable amount of free labeled amino acid available, nor any further increase in the amount of radioactive protein in the ganglion cells. More important than the changes in the amount of radioactivity in the nerve, however, was the fact that the distribution of radioactive protein along the nerve was changing with time. At any time after the isotope was injected, this distribution, beginning at a point about 0.5 mm behind the eyeball, could be described by a straight line on a semilogarithmic plot (*11*). As the interval after the injection increased, the slope of this line gradually changed (Fig. 1) from an initially steep negative value, through zero, to a positive value, and then to zero again as the overall radioactivity fell. This sequence of changes is consistent with the idea that a wave of labeled protein like that described by Taylor and Weiss for the mouse eye (*5*) is being transported down the nerve, but that the length of the wave is long, compared to the length of nerve being examined. Since the wave is so spread out, it is difficult to determine its velocity, but one may get an idea of the rate involved by making two assumptions. One assumption is that when the slope of the logarithmic distribution first becomes zero [for example, between 8 and 16 days (Fig. 1)], the peak of the wave is centered in the length of nerve under examination; that is, it has reached a point about 2 mm behind the eyeball. The other is that the average distance the wave has traveled within the eye (eye diameter 6.5 to 7 mm) is about 3 mm, giving a total transport distance of 5 mm. In a series of 31 animals killed at selected intervals after injection, the average time taken for the slope to flatten out was 12 days. Thus the average velocity must be about 0.4 mm per day. This rate is somewhat higher than, but still of the same order

as, that calculated by Edström (0.1 mm per day) for the displacement of radioactivity maxima in an analogous experiment on isolated Mauthner's cell axons from goldfish (*6*).

Another way of determining the rate of transport, which confirmed the validity of the order of magnitude of the above measurement, was to examine the rate of increase of radioactivity at various points along the nerve. For example, the time for the radioactivity to rise to half maximum differed by 2½ days at points 2 mm apart on the nerve, giving a transport rate (which would be expected to be about twice average) of 0.8 mm per day. Furthermore, when the optic fibers were examined after they had entered the optic tectum, that is, at a point about 10 mm away from the eye, the radioactivity in them increased appropriately slowly. Silver grain counts made from radioautograms of the tectum showed that in the fiber layer of the tectum there was a large increase in radioactivity beween 10 and 25 days after the injection (Fig. 2A). This indicates that the peak of the wave of radioactivity was arriving at some time later than 10 days, which confirms that the average rate of movement of the labeled protein was less than a millimeter per day.

However, the same radioautograms showed that, as early as 24 hours after the injection, there was already an accumulation of radioactivity in the tectum, concentrated in a single band 50 to 100 μ thick, lying about 50 μ from the surface (Fig. 3B)(*12*). The following facts indicate that this radioactive protein had been conveyed to the tectum by the optic nerve, and that it was located in the presynaptic optic fibers and not in the postsynaptic cells or glial cells. (i) If the optic tract had been cut before the injection, no localized band of radioactivity was seen. The denervated tectum showed only diffuse light labeling, which appeared as early as 4 hours after the injection, presumably caused by the leakage of labeled amino acid from the eye and its subsequent uptake by the cellular elements of the tectum. After 2 to 2½ weeks the localized band of radioactivity appeared, as the previously labeled optic fibers regenerated. (ii) If the optic nerve was cut after the radioactivity had become localized in the tectum, the band of radioactivity disappeared in 5 to 6 days, and over the same period there was a substantial reduction in the number of optic fibers seen in silver-stained sections of the tectum. (iii) Silver grains were almost never seen over the nuclei and only rarely over the cytoplasm of the nerve cell bodies.

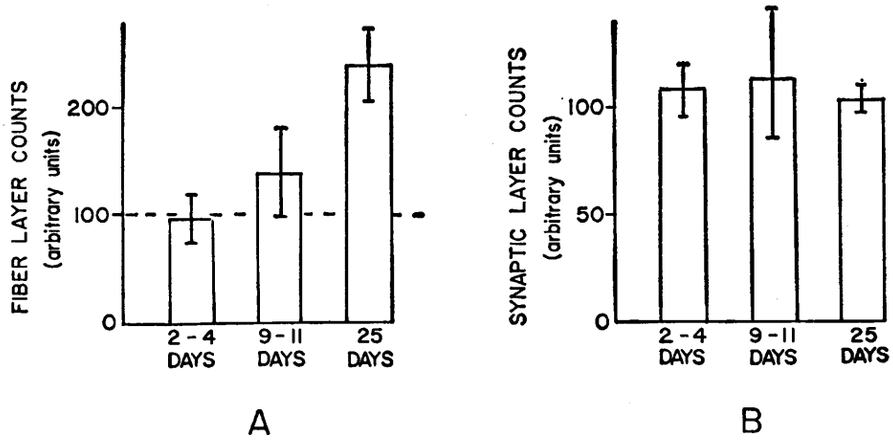

Fig. 2. Grain counts in radioautograms of optic tectum, showing level of radioactive protein (standard deviations indicated). (A) Counts over layer containing compact bundles of optic fibers (11 animals). These counts were made near the lateral or medial margins of the tectum, where this layer is thickest. (The dotted line indicates the level of radioactivity in the fiber layer of the tectum of the opposite side, in which the optic tract had been cut prior to the injection. This is considered 'background level.' Ordinate: counts as percentage of background level.) (B) Counts over layer containing synaptic endings of optic fibers (22 animals). (Ordinate: counts as percentage of average of whole series.)

When the radioautograms were compared with silver-stained sections it was seen that the band of radioactivity was delimited by two parallel optic fiber layers (F_1 and F_2 in Fig. 3A), and that it extended over a region which Leghissa (*13*) identified, at least in part, as containing the synaptic endings of the retinal fibers (*14*). This region is also

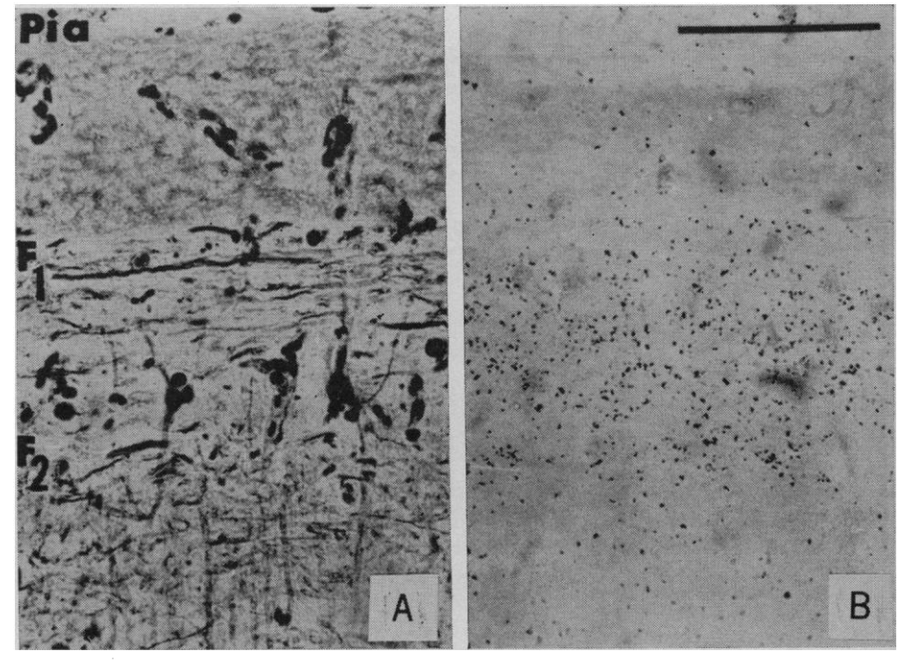

Fig. 3. Corresponding regions of tectum in (A) silver-stained section (modified Bodian protargol) and (B) radioautogram (lightly stained with Cason's stain) of animal killed 48 hours after injection of label into opposite eye. Section thickness 6 μ. F_1 and F_2 indicate the location of the principal parallel optic fiber layers. Note the accumulation of grains in the region delimited by these two layers. The level of radioactivity outside this region was identical to that in the tectum of the opposite side, where the optic tract had been cut prior to injection. The horizontal bar is 50 μ.

the site of generation of extracellularly recorded negative postsynaptic potentials evoked by visual stimulation (*15*). What we are probably dealing with, therefore, is the accumulation of radioactivity in the synaptic endings of optic fibers. Weiss and Holland have shown that an analogous accumulation of radioactive protein occurs in amphibian olfactory fiber endings, whose characteristic glomerular structure can be traced out in the arrangement of the silver grains in radioautograms of the olfactory bulb (*5*).

What is especially significant is that the increase of radioactivity in the synaptic region did not follow that in the nearby fiber layer which contains the main trunks of the optic axons. Two days after the injection, before the radioactivity in this fiber layer had risen above background level (Fig. 2A), the amount in the synaptic layer was already at its maximum and remained approximately constant for at least another 23 days (Fig. 2B), whereas the amount in the fiber layer was steadily increasing over the same period. To explain these findings, it can be postulated that (i) there is a special rapid transport of protein from the cell body to the synaptic endings, at a rate of at least 10 mm per day; (ii) this protein does not mix to an appreciable extent with the more stable proteins of the body of the axon; (iii) either this protein has an extremely long half-life in the endings, considerably in excess of the 25-day observation period, or, what is more likely, as it decays other labeled protein fractions arrive from the axon at a rate adequate to maintain a constant level of radioactivity in the endings. In this case, the half-life of the rapidly-transported protein, as determined from the figures on the rate of increase of radioactivity in the fiber layer, would appear to be about a week.

Miani has already emphasized (*16*) that some materials may be transported down axons at very rapid rates, and Lubinska (*3*) has suggested that this rapid movement might be due to cytoplasmic streaming. However, since the rapidly moving protein component described here apparently does not become dispersed in the axonal cytoplasm, this explanation does not seem adequate. Since the rate of transport involved is of the same order as that observed by Weiss *et al.* (*17*) for extracellular flow of materials within the nerve sheath, this is a transport route that must be considered, although it would be necessary to postulate that there is a mechanism, existing only at the axon terminals, for reabsorbing the extracellularly conveyed protein. A more satisfactory hypothesis is that the transport of the rapidly moving protein is confined to special structures in the axoplasm, which occupy only a small proportion of the axon cross section (*18*). For example, if soluble protein were involved, these structures might be liquid channels of the kind that Weiss has proposed (*19*). However, it is equally possible that the protein is directly incorporated into cell organelles or particles, which are able to move at rapid rates through the axonal matrix (*3*).

On the other hand, the slowly moving protein component is probably incorporated into the main body of the axoplasm, and may possibly be propelled by peristaltic movement of the axon (*5, 19*). The rate of this movement, 0.4 mm per day, is low compared to the values of 1 to 5 mm per day usually observed in mammalian nerves (*3*), but the difference could readily be explained by the 15°C temperature difference. What has previously been pointed out (*3, 7*) is that the rate of transport usually corresponds to the rate of regeneration of the fibers involved. This is also approximated in the present case, since a value of 0.2 mm per day has been obtained for the rate of regeneration of fish optic fibers (*20*). In the regenerating fibers themselves, however, the protein transport rate was 0.8 mm per day, twice as high as normal (*21*). It seems likely therefore that the slow transport mechanism serves to maintain and renew structural components of the axon.

By contrast, the rapid transport mechanism, operating at least 25 times as fast, appears to be more suitable as a special communication system between the cell body and nerve endings, and one might expect that the rate of movement of materials in this system would be more sensitive to alterations in the functional state of the neuron. It is also possible to imagine that this same system might serve as a means of communication in the reverse direction, from endings to cell body, if we assume that the transport results primarily from the utilization of materials by the nerve endings. As a rough analogy, the materials might be thought of as being "sucked down" by the endings rather than "pushed out" by the cell body. With such a model, it would be easier to explain how different substances might simultaneously travel down the axon at different velocities (*16*) which would be determined by their respective rates of utilization in the endings. It would also be easier to explain how the "information" about a requirement for increased protein synthesis for regeneration might be rapidly conveyed to the cell body when its axon is damaged.

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11. The points in the initial 0.5 mm could be described by a separate straight line whose slope flattened slightly during the first 3 days after the injection, then remained constant. It is most probable that this segment of the distribution represents direct uptake of radioactive amino acid by glial cells and other nonneuronal components in the optic nerve head.
12. This early accumulation of labeled protein has also been found by B. S. McEwen (personal communication) and by H. Rahmann (personal communication to P. Weiss).
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14. The layer here identified as F_2 *disappears* when the optic nerve is cut. This was apparently not recognized by Leghissa since he seemed to believe that this layer consisted primarily of intratectal elements. This may also account for his not having recognized the full extent of the region containing the synaptic endings of the optic fibers.
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