posed by the intracellular incorporation of plutonium.

Our results encourage further efforts to achieve quantitative electron-microscopic autoradiography. Preliminary measurements, based solely on geometric factors, indicate not only that quantitative EM autoradiography is feasible, but that the fraction of plutonium activity seen as tracks is approximatey the same (about 2 percent) as that in autoradiograms prepared for optical microscopy (*3*). Improvements in methodology, aimed at increasing the fraction of plutonium activity seen as alpha tracks, would be helpful. For example, an increase in the sensitivity of the photographic emulsion (*10*) would permit either the visualization of more tracks or the use of lower amounts of injected radionuclide. Attainment of these objectives would permit calculation of the irradiation energy delivered by a deposited radionuclide to tissues and organelles within a radius of about 25 μm. Such microdosimetric information could also be used to determine the differential reactions of specific tissue components to various amounts of irradiation by internal emitters.

The deposition of plutonium in liver and spleen appears to be a function of particle size; the critical particle diameter is estimated to be 0.5 to 0.01 μm (*11*). This preferential retention of particulate matter in liver, spleen, and bone marrow is thus of both practical and theoretical interest. Looking beyond the toxicological aspects toward the elucidation of cellular functions in these organs, we may consider the alpha activity of plutonium simply as a label that permits investigation of the behavior of colloids and macromolecules in living tissues.

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Electron Microscopic Radioautography: Identification of Origin of Synaptic Terminals in Normal Nervous Tissue

Abstract. *A method combining intraocular injection of tritiated leucine and electron microscopic radioautography has been used to study the distribution of optic nerve terminals in the monkey lateral geniculate nucleus. Radioactive label is found in only one type of synaptic terminal, which demonstrates that this terminal is of retinal origin and that this approach can be used to study the origin of synaptic terminals in normal tissue.*

In 1948 Weiss and Hiscoe (*1*) proposed that in the nervous system the principal site for biosynthesis of macromolecules is the cell body of the neuron and that these molecules subsequently flow or are transported along the axon. After the injection of tritiated amino acid into a specific neuronal site, the isotope is incorporated into protein within the neuronal cell body (*2–5*). This protein then moves along the axons that originate from the neurons in the injected region, finally appearing in their terminal portions. Two rates of this axonal movement have been reported, a "rapid" rate exceeding 40 mm/day and a "slow" rate of 1 mm/day (*6*).

When this sequence of events is followed by light microscopic radioautography, the result is a sensitive neuroanatomical tracing method for normal fiber systems (*7*). The axons originating from the injected site are localized and their distribution is demonstrated with a sensitivity that compares favorably with, and in some cases surpasses, the results obtained by Nauta degeneration methods. It would be desirable to extend this method to include the use of electron microscopy since, at the present time, determining the origin of synaptic terminals involves making a specific lesion and then identifying which terminals subsequently degenerate. Although such degeneration methods are useful, they have their limitations and ambiguities of interpretation (*8*).

Certain questions must be answered about the axonal movement of labeled protein before it can be studied with the electron microscope. Does the labeled material reach the synaptic terminal? If it does, what synaptic structure contains the label? Is there any movement of labeled material across the synaptic gap into the postsynaptic process?

[4-5-^{3}H]-Leucine (50 to 150 μc; specific activity, 15,000 to 25,000 mc/mmole) in 0.1 to 0.2 ml of sterile water was injected aseptically into the vitreous cavity of the left eye of monkeys (*Macaca mulatta*), weighing 2 to 3 kg. The monkeys were killed 3 to 30 days after injection by intravascular perfusion with phosphate-buffered 4 percent paraformaldehyde (*9*). The brain was removed, and samples of tissue from retina to visual cortex were postfixed in 2.5 percent osmium tetroxide in phosphate buffer (*p*H 7.4), dehydrated, and embedded in Epon. Thin sections were coated with Ilford L4 photographic emulsion by the method of Granboulan (*10*). After ex-

Table 1. Electron microscopic localization of silver grains in the lateral geniculate nucleus 21 days after intraocular injection of tritiated leucine.

Cell structure	Percentage of total
Axons	
Mitochondria	7
Axoplasm	17
Synapses	
Large dense profiles (type 1)	
Mitochondria	23
Axoplasm containing synaptic vesicles	33
Axoplasm containing neurofilaments	4
Small pale profiles (type 2)	2
Small dense profiles (type 3)	0
Neurons including dendrites	5
Glia including myelin	7
Unidentified profiles	2

posure for 4 to 6 months the sections were photographically processed, stained with aqueous uranyl acetate and lead citrate (*11*), and examined in a Siemens Elmiskop I electron microscope.

After vitreal injection of tritiated leucine, the label rapidly appears in retinal proteins (*2*, *4*, *5*). Scintillation counting (*12*) of the monkey visual system after injection with tritiated leucine indicated that subsequently some of this incorporated label moved along the optic nerve. A small amount of rapidly moving labeled component reached the lateral geniculate nucleus (LGN) and superior colliculus (SC) 3 days after the injection, indicating a minimum rate of 10 mm/day. Most of the labeled material moved at the rate of 1 mm/day, reaching the optic chiasm 13 to 15 days and the LGN and SC 26 to 30 days after injection. Chemical studies show that after an intraocular injection there is little or no diffusion of free leucine along nerve (*2*, *4*, *5*). Furthermore, in electron microscopic radioautographs of the left optic nerve from these monkeys, 93 percent of the silver grains are localized within the axon, with 16 percent over mitochondria and the rest over the axoplasm (*13*).

Light-microscopic radioautography of the LGN (Fig. 1) showed a laminar distribution of silver grains corresponding with the optic axon distribution previously determined by degeneration methods (*14*); that is, the ganglion cells of the left retina send axons to laminae 2, 3, and 5 of the left LGN and laminae 1, 4, and 6 of the right LGN. However, by light microscopy it was not possible to be certain that the label had reached the actual synaptic terminal since electron microscopy has shown that in the monkey LGN incoming axons terminate mainly on the dendrites, often at some distance from the neuronal cell bodies (*15*, *16*). For the same reason localized transynaptic movement of label into dendrites could not be ruled out in these radioautographs.

With the electron microscope three types of LGN terminals have been identified on the basis of their morphological differences (*15*, *16*). After fixation in paraformaldehyde and osmium, type 1 is large (2.5 to 6 μ) and contains numerous mitochondria with few cristae and irregular outer membranes. The terminal cytoplasm is characteristically dense and contains numerous round synaptic vesicles (500 Å) as well as an occasional larger (800 to 1000 Å) dense-cored vesicle and a few neurofilaments. This terminal makes multiple presynaptic contacts with both soma and dendrites of LGN neurons as well as with another type of axon terminal. These type 2 terminals are smaller (1 to 3 μ), have small dark mitochondria that stand out against the pale cytoplasm, and contain flattened or tubular synaptic vesicles. They are presynaptic to other type 2 terminals, dendrites, or the neuronal cell body. Type 3 is small (0.5 to 1.5 μ), has few mitochondria, and is densely packed with round, 500 Å synaptic vesicles. Type 3 most frequently synapses on small dendrites or type 2 terminals; it is

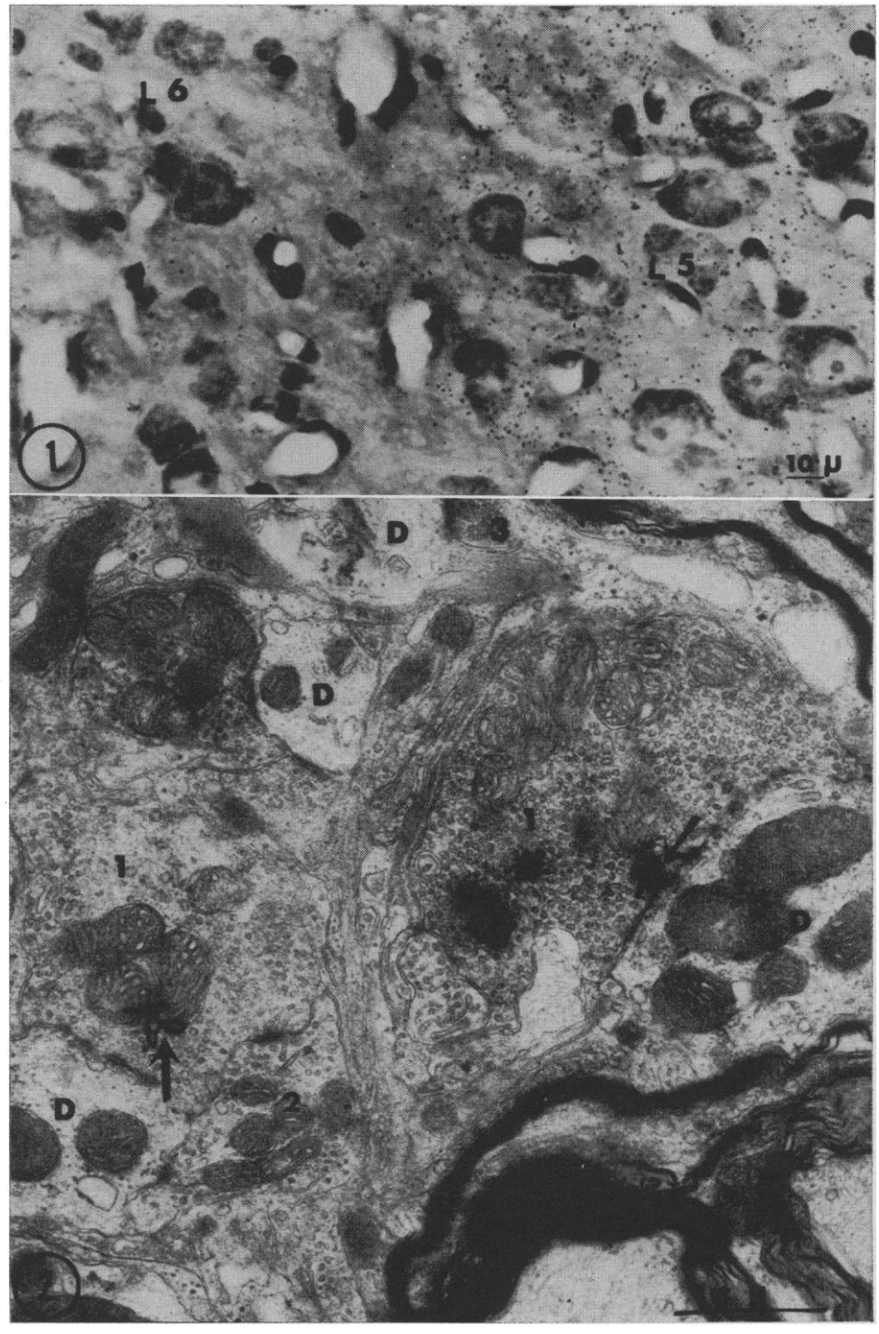

Fig. 1. A light microscopic radioautograph of the left LGN from a rhesus monkey 28 days after injection of tritiated leucine into the left eye. Lamina 5 (*L5*) receives axons from the left retina and contains numerous silver grains around the neuronal cell bodies. The same regions in lamina 6 (*L6*) contain only scattered silver grains. The labeling over neuronal cell bodies is the same in both lamina (× 560). Fig. 2. An electron microscopic radioautograph from a rhesus monkey LGN 21 days after intraocular injection. A synaptic area shows the types of synaptic terminals: the large, dense type 1 (1), the smaller pale type 2 (2), and the small dense type 3 (3). Two of the type 1 terminals contain silver grains (arrows) over both vesicle-rich regions and mitochondria. The other two terminal types and the dendrites (*D*) are not labeled (× 20,000).

not synaptically related to the type 1 terminal. Since type 1 shows first a neurofilamentous degeneration reaction and then disappears after the optic nerve is cut, it apparently originates from the retinal ganglion cell. Since the other types of terminals are reduced in number after optic nerve section, there is a possibility some of them also are of retinal origin (*15*, *17*).

Electron microscopic radioautographs of LGN laminae receiving radioactive axons revealed that tritium-containing material did reach the synaptic terminals (Fig. 2). Of the three synaptic types, only type 1 was definitely labeled, containing 60 percent of the 139 silver grains counted in the LGN (Table 1). Three grains were found over the cell membranes of an adjacent type 1 and type 2 terminal, but since the resolution in these radioautographs is 2000 Å (*18*) there is an equal possibility that these grains originated from a radioactive decay occurring in either terminal. Within the type 1 terminals the greatest number of silver grains (33 percent) was over cytoplasmic areas rich in synaptic vesicles, but the resolution prevented actually localizing them to the vesicles themselves. Mitochondria also contained numerous grains (23 percent), whereas the bundles of neurofilaments within the terminal were infrequently labeled (4 percent).

Light microscopic radioautographs indicated that the numbers of silver grains over the LGN neuronal cell body did not vary between laminae receiving radioactive and nonradioactive axons. In electron microscopic radioautographs, although silver grains were found over synaptic vesicles adjacent to the membrane density that apparently marks the site of synaptic interaction, there was no concentration of grains over the dendrites opposite this density. As judged from grain counts, labeling of neurons (5 percent) was little different from that of glia (7 percent) in the LGN, which indicates that neuronal labeling is caused by blood-borne tritiated leucine and not by a transynaptic movement of axonal labeled materials. This is in contrast to a previous light microscopic radioautographic study of peripheral nerve which suggested a transynaptic movement of protein labeled with C^{14}-glycine from hypoglossal nerve into tongue muscle (*19*).

Both a "rapid" and a "slow" rate of axonal movement has been shown in the visual system (*2*, *4*, *5*, *12*). Since it has been reported that substances differing in particle size and chemical composition constitute these two components in the goldfish visual system (*5*), the distribution of label within the synaptic terminal might be different at shorter or longer survival times. The observation that regions rich in synaptic vesicles contain the greatest amount of label indicates that at least some component associated with these vesicles originates in the neuronal cell body. What axonal structures contain this labeled material and at what point the vesicles appear as morphological entities is still not clear. In this connection, the small amount of label over cytoplasmic regions containing neurofilaments within the terminal axon is interesting since both neurotubules and neurofilaments have been implicated in axonal transport (*20*). The observation that both axonal and terminal mitochondria contained silver grains is understandable since these organelles synthesize protein (*21*) and move between the cell body and the axon (*22*).

These data indicate that after an intraocular injection of tritiated leucine, tritiated material is contained within the axons originating from the injected eye and is distributed throughout their extent, including the synaptic terminal. In the LGN only the type 1 terminal is definitely labeled; thus it originates from the retina. The two nonlabeled synaptic terminal types apparently originate elsewhere. The labeled material does not cross the synaptic gap into dendrites; neither does it appear to cross an axo-axonal synapse.

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Hormonal Induction of Increased Zinc Uptake in Mammalian Cell Cultures: Requirement for RNA and Protein Synthesis

Abstract. *The zinc content of HeLa S_3 cells is markedly increased after growth in medium containing adrenal glucocorticoid hormones. Studies with inhibitors indicate that the synthesis of RNA and protein is required for enhanced zinc uptake. When protein synthesis is blocked in the presence of the steroid, an intermediate, presumably messenger RNA, which specifies enhanced zinc uptake accumulates and is expressed when the inhibition of protein synthesis is removed.*

Hormones have been implicated in the control of the ionic content of cells, and the ensuing alteration in the intracellular environment may explain, in part, the effects of a hormone on a particular cell or tissue (*1*). Adrenal glucocorticoid hormones increase the uptake of zinc in certain mammalian cell cultures (*2*). This enhanced accumulation of zinc is specific in that the uptake of other cations—for example, calcium and rubidium—is not altered and a number of other monovalent and divalent ions do not compete with zinc for up-