Table 1, because no control group of simultaneously sensitized animals was included. Rapid recovery from paraplegia occurred in all seven animals. They appeared clinically well from day 18 to day 23 after sensitization, when they were killed.

Histological studies on animals in three experiments confirmed the clinical observations. Of 19 rats treated with cyclophosphamide, eight had no demonstrable microscopic lesions of EAE and nine were scored 1+. Only two were scored as 2+. None of the 24 saline-treated or nontreated rats were free of EAE lesions. Two were scored 1+, and 8 as 2+, and 14 as 3+.

Our findings provide additional evidence of the potent immunosuppressive actions exerted by cyclophosphamide in averting tissue damage that occurs in EAE (*4, 5, 13, 14*), allergic thyroiditis (*15*), and allograft rejection (*16*). We believe this is the first report of complete reversal of an experimental autoimmune disease initially treated with an immunosuppressive drug several days after "full-blown" clinical manifestations of the disease have appeared. Brandriss (*17, 18*) reported clinical improvement of guinea pigs with moderately severe EAE (more than 50 percent being paralyzed by day 13) treated with methotrexate, starting on the 1st day of EAE signs. In other experiments with animals developing more severe EAE (more than 50 percent paralyzed by day 10), methotrexate had little, if any, influence on the course of disease. While 6-mercaptopurine, chlorambucil, nitrogen mustard, and x-irradiation have been reported to inhibit development of EAE, none has been shown capable of interrupting the course of this disease if administered after onset of disease (*3, 19*).

Whether the preventative and therapeutic effects of the drug reside in a common mechanism or different mechanisms of action is not known. The preventative effects have a firm immunologic basis (*5, 14*). There are insufficient data to permit any statement now about the therapeutic action of the drug.

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High-Resolution Autoradiography of Intracellular Plutonium

Abstract. *Intracellular incorporation of polymeric plutonium injected into mice was demonstrated in liver and spleen by electron-microscopic autoradiography. The observations are not inconsistent with other evidence indicating the association of plutonium with lysosomal components. Early results with a quantitative electron-microscopic technique may lead to microdosimetry of cells and cellular components.*

The projected use of increasing amounts of plutonium and transplutonium elements in reactors, mobile power sources, and so forth, may be expected to increase the incidence of human contamination by these highly toxic radionuclides. Nuclides of the transition and actinide series tend to hydrolyze and polymerize under physiological conditions. For this reason their deposition in tissues, in addition to being a function of their chemical properties, is complicated by the biological process of phagocytosis. Although there are some differences in the physiological behavior of these elements, plutonium-239 may be considered to be a prototype of this class of radionuclides. Metabolic and therapeutic studies have shown that the hazard of this radionuclide is, in part, related to the long-term retention of particulate plutonium in liver, spleen, and bone marrow (*1*). A large fraction of this plutonium is inaccessible to the action of chelating agents such as diethylenetriaminepentaacetic acid (DTPA) (*2*). To devise effective therapeutic procedures, therefore, it is important to determine, as exactly as possible, the mode of plutonium deposition in these organs.

The usefulness of autoradiography, combined with electron and light microscopy, has been investigated (*3, 4*), and in the latter a quantitative technique for the assay of plutonium deposited in specific areas of animal tissues has been demonstrated. Particulate plutonium administered in the form of PuO_2, as demonstrated by electron microscopy (EM), is incorporated in pulmonary macrophages after administration as an aerosol (*5*) and in peritoneal macrophages removed after intraperitoneal injection of a saline suspension (*6*). Quantitative autoradiography with the electron microscope would permit radionuclide microdosimetry in tissue regions occupied by intracellular components.

Most EM autoradiographic studies have employed tritium, which emits beta particles of low energy (0.018 Mev maximum). In such preparations the reduced silver grains are formed nearly exactly above the deposited radionuclide. However, with nuclides that emit alpha particles of relatively high energy (about 5.15 Mev in the case of plutonium-239), the relationship between grain and source extends over a longer distance, and the grains (seen either singly or arranged as "tracks" made up of five or more aligned grains) may be formed as far as 25 to 30 μm from the source. Two or more tracks with a common origin form a "star." A grain or track in the photographic emulsion represents only a small fraction of the total alpha radiation delivered to the surrounding tissue by the embedded nuclide.

Although it is nearly impossible, in cells or cellular organelles, to locate deposits containing plutonium by the appearance of isolated grains, the convergence area of two or more tracks should

be located over the plutonium source. Frequently in EM autoradiograms a cluster of single grains in the emulsion and an electron-opaque mass may be seen in this area. The feasibility of counting alpha tracks at such high magnification is dependent upon the introduction of sufficient radionuclide into the tissues to produce usable autoradiograms within a reasonable exposure time (6 months or less) without also causing serious radiation damage. To investigate the severity of these limitations, we injected mice intravenously with increasing amounts of polymeric [23 percent ultrafilterable (*7*)] plutonium-239, prepared from a solution of $Pu(NO_3)_4$ adjusted to *p*H ~7. Mice given 93 microcuries per kilogram of body weight survived for at least 19 days before exhibiting gross toxic symptoms; liver and spleen removed 4 days after administration of the plutonium showed only slight cellular damage, with no alteration in extracellular organization. These tissues were used to prepare the autoradiograms described below. Sections about 500 Å thick were fixed with osmium tetroxide, stained with lead citrate and uranyl acetate, and overlayed with Ilford L-4 photographic emulsion (*8*). Exposure time was 97 days.

The EM autoradiograms demonstrate intracellular plutonium *in situ* in liver and spleen. Although they show the incorporation of plutonium within membrane-bound intracellular structures, they provide no clear-cut demonstration that plutonium is associated with a specific intracellular site or organelle. However, other evidence, based on radiochemical and enzymatic analysis of liver homegenates fractionated by a gradient centrifugation technique (*9*), strongly indicates that the uptake of plutonium is, at least partially, lysosomal. These autoradiographic results confirm the findings of Sanders *et al.* (*5*, *6*). They furnish, in addition, evidence for the long-held assumption that the ineffectiveness of DTPA in removing plutonium from tissue such as the liver is the result of permeability barriers im-

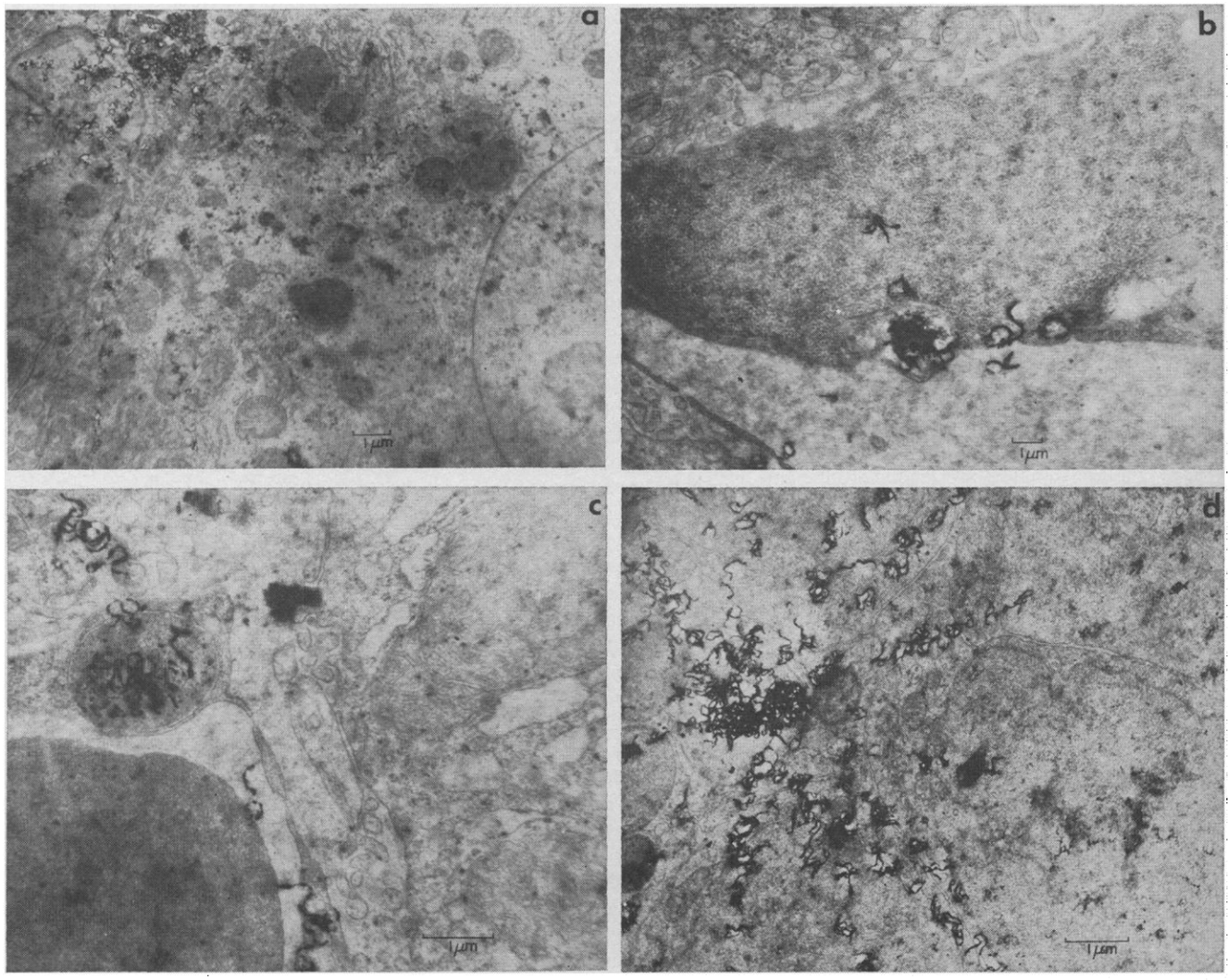

Fig. 1. Autoradiograms of plutonium in mouse liver and spleen. (a) Portion of a typical star in liver. The appearance of the tracks and of the cluster of silver grains above a somewhat abnormal cytoplasmic structure within a littoral cell indicates the diffuse nature of the deposited plutonium. A 12-μm portion of one track, demonstrating alpha irradiation of both cytoplasmic and nuclear portions of an adjoining parenchymal cell, may be noted. (b) Sinusoidal area in liver, showing a portion of a littoral cell, apparently in the process of incorporating a body containing plutonium from the circulation. (c) A structure containing aggregated plutonium may be seen in an endothelial cell of a liver sinusoid. The large dark mass within the sinusoid is a portion of an erythrocyte. (d) An EM autoradiogram of mouse spleen. A star appears to be centered above a mass of intracellular plutonium.

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