DISSERTATION

LYMPH NODE CLEARANCE OF PLUTONIUM FROM SUBCUTANEOUS WOUNDS IN BEAGLES

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

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COLORADO STATE UNIVERSITY

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ABSTRACT OF DISSERTATION

LYMPH NODE CLEARANCE OF PLUTONIUM FROM SUBCUTANEOUS WOUNDS IN BEAGLES

The lymph node clearance of plutonium oxide from subcutaneous implants was studied in adult beagles to simulate accidental contamination of hand wounds. External in situ scintillation data were collected from the popliteal lymph nodes of each dog after 9.2 to 39.4 μCi of plutonium oxide was subcutaneously implanted into the left or right hind paws. The left hind paw was amputated 4 weeks after implantation to prevent continued deposition of plutonium oxide particles in the left popliteal lymph node. Groups of 3 dogs were sacrificed 4, 8, 16, and 32 weeks after plutonium implantation for histopathologic, electron microscopic, and radiochemical analysis of regional lymph nodes. An additional group of dogs received treatment with the chelating agent diethyenetriaminepentaacetic acid (DTPA).

Plutonium rapidly accumulated in the popliteal lymph nodes after subcutaneous injection into the hind paw, and 1% to 10% of the implant dose was present in the popliteal lymph nodes at the time of necropsy. Histopathologic changes in the popliteal lymph nodes with plutonium particles were characterized primarily by reticular cell hyperplasia, increased numbers of macrophages, necrosis, and fibroplasia. Eventually, the plutonium particles became sequestered by scar tissue

that often replaced the entire architecture of the lymph node. Light microscopic autoradiographs of the popliteal lymph nodes showed a time-related increase in number of alpha tracks per plutonium source. Electron microscopy showed that the plutonium particles were aggregated in phagolysosomes of macrophages.

There was slight clearance of plutonium from the popliteal lymph nodes of dogs monitored for 32 weeks. The clearance of plutonium particles from the popliteal lymph nodes was associated with necrosis of macrophages. The external iliac lymph nodes contained fewer plutonium particles than the popliteal lymph nodes and histopathologic changes were less severe. The superficial inguinal lymph nodes of one dog contained appreciable amounts of plutonium. Treatment with diethylenetriaminepentaacetic acid (DTPA) did not have a measurable effect on the clearance of plutonium from the popliteal lymph nodes.

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CHAPTER I

INTRODUCTION

Plutonium is increasingly used as a fuel in nuclear reactors. As discussed in recent reviews by Cook (1973) and Roberts (1973), over 95% of our energy sources came from fossil fuels as recently as 1969. With increasing energy demands the fossil fuels and fuels for nuclear fission from natural resources are rapidly being depleted. Although greater utilization of other energy sources, such as nuclear fusion, geothermal energy, solar energy, and tidal power may eventually occur, much of our future energy demand will probably be supplied by nuclear fission of elements such as plutonium.

Many production workers have been accidentally contaminated with plutonium and, with increasing use of plutonium in nuclear fuels, the incidence of occupational contamination will probably increase. The toxicity of plutonium is related to the route of contamination (inhalation, injection, ingestion, and cutaneous absorption), the chemical form, and the particle size (Thompson, 1967). A common route in man is through contamination of wounds of the hands (Norwood, 1972).

In several studies at Colorado State University (Johnson, 1969; Watters and Lebel, 1970; Lebel and Watters, 1972; Bistline, et al., 1972), the regional lymph nodes were found to be a major site of

plutonium in beagles after subcutaneous injections simulating contamination of hand wounds. An increasing amount of plutonium accumulated for 2 to 4 weeks in the left superficial cervical lymph nodes of dogs with plutonium implanted into the left forepaw. At longer durations decreasing amounts of plutonium were sometimes observed in the superficial cervical lymph nodes. The kinetics of lymph node clearance were complicated by continuous plutonium translocation to the superficial cervical lymph nodes from the injection site. The influence of the lymph nodes on the systemic deposition of plutonium from subcutaneous wounds was also difficult to assess because of the presence of variable numbers of superficial cervical lymph nodes draining the forepaw injection site (Gomez, 1972).

The present study was designed to investigate a potential biological model for studying the clearance of plutonium from wound sites, from lymph nodes and from phagocytic cells. Subcutaneous injections of PuO_2 were made in the hind paw of beagles to simulate puncture wounds in the human hand. The hind paw is more analogous to the human hand than the front paw, and the chain of lymph nodes in the hind leg of beagles can more readily be separated. Particles injected subcutaneously into the hind paw are transported through the lymphatics to the popliteal lymph node, from there to the external iliac lymph node and eventually to the systemic organs. After significant concentrations of plutonium were detected in situ with a wound counter, the hind paw was amputated. At selected time intervals after amputation, the popliteal

and external iliac lymph nodes were examined for histopathologic and ultrastructural changes.

The purpose of this study was to characterize the translocation of plutonium to and from the popliteal lymph node and to evaluate the histopathologic and ultrastructural changes that may influence translocation. Since plutonium accumulates in the lymph nodes, it is probable that phagocytosed particles of plutonium are retained by macrophages in the lymph node. The clearance of plutonium particles from the lymph node was hypothesized to result from necrosis of macrophages that have engulfed plutonium particles. In this way, plutonium particles could be returned to an extracellular position and carried into the efferent lymphatics. Alternatively, exocytosis of plutonium particles from macrophages could occur. These aspects of cellular kinetics were studied, as well as changes in the distribution of plutonium particles in the lymph nodes as functions of time and particle size. Possible changes in cellular kinetics of plutonium clearance caused by the chelating agent diethylenetriaminepentaacetic acid (DTPA) were also studied.

CHAPTER II

REVIEW OF LITERATURE

Early History of Plutonium

The importance of plutonium was recognized almost as soon as its discovery in 1941 (Seaborg, 1972). The potential energy of fission was appreciated at that time; and when plutonium was shown to maintain a chain reaction, full scale production was implemented. The only other substance available for fission was 235-uranium, and the separation of 235-uranium from 238-uranium involved difficult physical methods which took advantage of the relatively small difference in the mass of the two isotopes. Plutonium was separated from uranium during the manufacturing process by simpler chemical means. A large plant for plutonium production was started at Hanford, Washington in 1943. With war as a national priority, enough plutonium was produced by 1945 for the first atomic bomb.

Physical and Chemical Properties of Plutonium

Plutonium is a transuranic element that is produced artificially. The basic reactor process for plutonium production involves bombardment of ^{238}U with neutrons to produce ^{239}U. Neptunium and then plutonium are produced after beta decays. The fissionable plutonium

isotope of major importance, 239-Pu, has a half-life of about 24, 360 years and emits 5.1 mev alpha particles. One microgram of 239-Pu is equivalent to 0.064 microcuries or about 1.4×10^5 alpha disintegrations per minute. The various radionuclides commonly found in reactor-produced plutonium (Johnson, 1969) are shown with some of their properties in Table 1. Although 239-Pu is primarily an alpha emitter, reactor-produced plutonium contains a variety of isotopes with alpha, beta, gamma and X-ray emissions. One relatively short-lived isotope formed is ^{241}Pu ($T_{\frac{1}{2}}$=14 years) which has a daughter, ^{241}Am, present in nearly all production plutonium. Because ^{241}Am has an abundant yield of gamma rays (0.37 per alpha decay) of 59.6 kev, it is used as an in vivo tracer for ^{239}Pu to indicate biological contamination.

Plutonium belongs to the actinide series and its chemistry is similar to that of the rare earths (Wick, 1967). It melts at 640 degrees C and boils as 3327 degrees C. Plutonium is a highly reactive metal which oxidizes readily. Plutonium oxide is relatively insoluble and has several crystalline forms. Plutonium nitrate, plutonium citrate, and plutonium fluoride, other forms of plutonium that have been studied in laboratory animals, are soluble in biological systems. "Monomeric" plutonium is a term frequently used to indicate ionized or soluble plutonium particles, and "polymeric" generally refers to larger plutonium particles that emit alpha stars on autoradiographs.

Table 1. Characteristics of Selected Pu and Am Isotopes

Isotope	Half Life (yr)	Radiations	Energy	Abundance (%)
^{238}Pu	86.4	α	5.495 MeV	72
		α	5.452 MeV	28
		U L X-rays	17 keV (ave.)	10.55
		γ	43.5 keV	0.038
		γ	99.8 keV	8×10^{-3}
		γ	153.1 keV	10^{-3}
^{239}Pu	24,390	α	5.16 MeV	88
		α	5.11 MeV	11
		U L X-rays	17 keV (ave.)	4
		γ	38.6 keV	0.007
		γ	51.6 keV	0.020
		γ	129.3 keV	0.005
^{240}Pu	6580	α	5.17 MeV	76
		α	5.12 MeV	24
		U L X-rays	17 keV (ave.)	10
		γ	45.28 keV	
		γ	103.6 keV	
^{241}Pu	13.2	Beta	21 keV	99.97
		γ	145 keV	1.6×10^{-4}
^{242}Pu	3.79×10^{5}	α	4.90 MeV	76
		α	4.86 MeV	24
		U L X-rays	17 keV (ave.)	10
^{241}Am	458	α	5.49 MeV	85
		α	5.44 MeV	13
		Np L X-rays	17 keV (ave.)	37.6
		γ	26.36 keV	2.5
		γ	43.44 keV	0.07
		γ	55.56 keV	
		γ	59.56 keV	35
		γ	99 keV	0.024
		γ	103 keV	0.019
		γ	106 keV	0.001
		γ	122 keV	0.002
		γ	125 keV	0.005
		γ	146 keV	2×10^{-4}

Biological Effects of Plutonium

Plutonium is one of the more toxic materials known to man based on its potential to produce tumors in animals (Stover & Jee, 1972). Tumor induction reported in beagles includes osteogenic sarcomas after intravenously administered plutonium citrate (Taylor, et al., 1972) and bronchiolo-alveolar carcinomas after inhalation with plutonium oxide (Bair, 1970). Bronchogenic carcinoma has been reported in the baboon after the inhalation of plutonium (Metiviev, et al., 1972) and fibrosarcoma was reported at the injection site of plutonium in laboratory animals (Brues, et al., 1947). A single case of lymphosarcoma was reported in a beagle after the subcutaneous implantation of plutonium oxide (Lebel, et al., 1970). Neoplasia due to plutonium has not been reported in man.

The two common methods of exposure in man are by inhalation and wound contamination (Norwood, 1972). Plutonium is poorly absorbed from the gastrointestinal tract or the skin. The maximum permissible body burden of ^{239}Pu (0.04 μ Ci) was established as "the maximum amount of ^{239}Pu which, when fixed indefinitely in the human body, has the same improbability of producing significant bodily injury as does 0.1 μ Ci of ^{226} Ra" (Langham, 1959).

Inhalation studies in the beagle have been reviewed by Bair (1970). For the purposes of this study, it is sufficient to note that plutonium is rapidly translocated to the tracheobronchial lymph nodes. The

concentration of plutonium in tracheobronchial lymph nodes following inhalation exposure is generally higher than that in lung, bone, or liver. Thomas (1965) showed that the concentration of insoluble materials, including plutonium, in pulmonary lymph nodes is related to the concentration of the material in the lung. The biological mechanisms of plutonium transfer from the lungs to the tracheobronchial lymph nodes have been difficult to study, in part due to the difficulties of intrathoracic surgery.

Subcutaneous injections of plutonium in previous studies at Colorado State University have been used to simulate wound contamination in man. Johnson (1969) showed a continued deposition of air oxidized plutonium in the superficial cervical lymph nodes after subcutaneous injection into the forepaw. He showed that the average percent of implant activity in these lymph nodes started to stabilize at 1 month, and averaged from 6.7% to 13.3% after 1 to 12 months. Bistline, et al., (1972) studied the translocation dynamics of plutonium movement to the superficial cervical lymph nodes from the paws of dogs injected with $Pu(NO_3)_4$ and high-fired PuO_2. The $Pu(NO_3)_4$ reached a maximum buildup in the superficial cervical lymph nodes after 10 days and gradually decreased throughout the year of study. The high-fired PuO_2 reached a maximum around 30 to 40 days post injection in the superficial cervical lymph nodes and gradually declined over the next 9 months. Gomez, et al., (1972) showed that by the surgical removal of the superficial cervical lymph nodes of dogs implanted in the forepaw

with high-fired PuO_2 there were higher levels of plutonium in the systemic organs.

Chelating agents, which bind metal ions, are commonly used in the therapeutic removal of internally deposited plutonium (Smith, 1972). A commonly used chelating agent, diethylenetriaminepentaacetic acid (DTPA), has been shown to promptly reduce levels of plutonium in the skeleton, liver, and other soft tissues in laboratory animals. Recently the liposome-encapsulation of DTPA has been shown to further increase plutonium removal, presumably by the intracellular introduction of the DTPA (Rahman, et al., 1973).

Structure and Function of Lymph Nodes

The main structural units of the lymph node are divided between the cortex and medulla, as described in standard textbooks (Yoffey, 1970). The cortex includes the follicles: (1) primary follicles consist of nodules of lymphocytes, (2) secondary follicles include germinal centers surrounded by lymphocytes, and (3) tertiary follicles consist of the remaining cortical pulp. The medulla is composed of medullary cords separated by medullary sinuses. The medullary cords, also called the medullary pulp, are composed of lymphocytes, plasma cells, reticular cells, and occasionally stem cells. The medullary sinuses are lined with littoral cells and contain a variety of cells, generally including a few lymphocytes and macrophages. Lymph and blood vessels traverse

lymph nodes. Blood monocytes and granulocytes are frequently seen in the medullary cords and sinuses.

Although lymph nodes have a variety of immunologic functions, the ability to filter particulate matter by phagocytosis is of primary interest in studying the translocation of plutonium from subcutaneous injection sites. Lymph flows to the lymph node in the afferent lymphatics which empty into the subcapsular sinuses. From the subcapsular sinuses lymph moves into the medullary sinuses, and finally out through the efferent lymphatics at the hilus (Yoffey, 1970). The popliteal lymph nodes of dogs (Drinker, 1934) are very efficient in filtering lymph. When the hind limbs in anesthetized dogs were perfused with a mixture of dog erythrocytes and streptococci, almost all the foreign material was found in the popliteal lymph node; very little was present in the external iliac lymph node.

Lymph nodes belong to the "reticuloendothelial" system, a term proposed by Aschoff (1924) for cells having in common the property of phagocytosis. The importance of phagocytosis was first emphasized by Metchnikoff (1892) to describe a process of engulfment of particulate matter by cells and represents the method by which particles are filtered from lymph in lymph nodes. The broader term, "endocytosis", is used to describe the process of engulfing particulate or liquid material by any cell, and includes the processes of phagocytosis and pinocytosis. The term "phagocytosis" has historical precedence over "endocytosis", and describes the process occurring with particulate matter.

Macrophages are large cells having the ability of phagocytosis (Metchnikoff, 1892). Spector (1969) reviewed the preponderance of experimental evidence indicating the bone marrow origin of macrophages. Macrophages are located throughout the lymph node; however, those macrophages filtering particulate material are closely associated with the lymph flow in sinuses. In light microscopic sections stained with hematoxylin and eosin, macrophages have a moderate amount of eosinophilic cytoplasm, a clearly delineated cytoplasmic membrane, and a round-to-ovoid and occasionally slightly indented large nucleus with evenly distributed chromatin and small nucleoli. The electron microscope shows a cell with fine cytoplasmic pseudopodia, numerous lysosomes, phagosomes, smooth and rough endoplasmic reticulum, several Golgi areas, and a large nucleus with finely distributed chromatin and small nucleoli.

Littoral cells (Morat, 1964), flattened phagosome containing cells that line sinusoidal spaces of the lymph node, also demonstrate phagocytic activity. They probably have less phagocytic activity than macrophages, and may act as a reserve cell when macrophages are overburdened. They have also been referred to as "retothelial cells" (Mori, 1969), "sinusoidal cells", "endothelial cells", or "modified reticular cells" (Mae, 1964).

Reticular cells form the net-like framework of the lymph node and give origin to other differentiated cells in the lymph node. Mae (1964) describes differentiation of primitive reticular cells into large

lymphocytes, plasma cells, cells which look like fibroblasts, and macrophages. Reticular cells have a large amount of cytoplasm with indistinct cytoplasmic borders and few phagosomes as observed with the light and electron microscopes. Mori (1969) refers to macrophages in lymph nodes as phagocytic reticulum cells, suggesting the transformation of reticulum cells into macrophages. Phagocytic reticular cells, which may or may not represent macrophages, phagocytose material and assist in the filtering capacity of lymph nodes.

The mechanisms of phagocytosis have been extensively studied (Dingle & Fell, 1969). A particle is first engulfed by a merging of pseudopods extending from the cell membrane. As the pseudopods merge, the particle comes to lie in a vacuole, called a "phagosome", that moves into the cytoplasm of the cell. This phagosome is formed from the cell membrane and is lined on its inner surface by the glycocalyx. It is considered relatively free of hydrolytic enzymes. The phagosome then merges with a lysosome to form a phagolysosome or secondary lysosome. Lysosomes are intracytoplasmic vacuoles, probably formed by the Golgi apparatus, that are filled with many hydrolytic enzymes including acid phosphatase. After phagolysosomes are formed, hydrolytic enzymes are available to digest the ingested foreign material. The phagolysosomes generally become residual bodies and frequently remain for the life of the cell. Exocytosis, the process of vacuoles being disgorged from a cell, has been described, but is unusual in mammalian systems.

Lymph nodes react to injury in predictable ways (Anderson, 1966). Acute lymphadenitis is characterized by edema, congestion, increased numbers of neutrophils, and increased numbers of monocytes in the lymph sinuses. Lymphoid tissue becomes hyperplastic with continuous antigenic stimulation, or atrophic in response to corticosteroid therapy or continued stress. Longer term injuries will lead to chronic lymphadenitis, characterized by greater numbers of mononuclear inflammatory cells and macrophages and, occasionally, fibrosis. Lymph nodes are also prone to develop granulomatous inflammation characterized by the infiltration of macrophages, epithelioid cells and occasionally multinucleated giant cells. Spector (1969) reviewed monocyte emigration, monocyte transformation into macrophages, cell division, cell turnover, and proliferation in granulomatous inflammation. In granulomatous inflammation there is an attempt to sequester poorly soluble irritants in macrophages, and there may be a natural selection of macrophages having a low turnover rate.

Effects of Plutonium and Other Sources of Ionizing Radiation on Lymph Nodes

The toxic effect of plutonium on lymph nodes is caused by the radioactivity emitted, primarily alpha particles, from plutonium that has translocated to the lymph node. The 5.1 mev alpha particles from plutonium penetrate soft tissues about 50 microns (Sanders and Adee, 1969). The physical form influences toxicity since the size of the

plutonium particle influences the concentration of energy release in the lymph node. It was reported that chemical form (Bistline, 1972) influences translocation dynamics to and from the lymph node.

The radiosensitivity of lymphoid tissue was recognized early (Warthin, 1906; Jolly, 1924). The histologic changes induced in lymphoid tissues of lymph nodes, spleen, and thymus after total body irradiation in the lethal range are similar for different animals and were summarized by Rubin and Casarett (1968). Within 1 hour there is extensive necrosis of lymphocytes and in a few hours there is marked reduction in numbers of lymphocytes in the lymphoid organs due to necrosis, movement of lymphocytes out of lymphoid organs, and cessation of proliferation. In 1 to 3 days the necrotic debris is removed from lymph nodes and spleen due to phagocytosis by fixed and free macrophages and flushing with lymph. Regeneration of lymphoid tissue begins within the first week in lymph nodes and spleen. The thymus, perhaps due to slower removal of necrotic debris, starts to regenerate a few days later. Engeset (1964) described a second wave of lymphoid atrophy after local irradiation of lymph nodes. This second wave started after 2 weeks and progressed slowly; it was associated with degeneration, thickening, and reduction in numbers of small blood vessels, and increased amounts of connective tissue. After 9 to 12 months the normal architecture of the lymph node was nearly completely obliterated.

Early ultrastructural studies on the effects of ionizing radiation on cells emphasized changes in nuclei such as chromatin derangement, focal chromatin condensation, margination of chromatin, and edema (Alexander and Bacq, 1961; Cottier, et al., 1963). Cytoplasmic ultrastructural changes predominated in lymph node cells, radioresistant mouse epithelial tumor cells, and radiosensitive mouse spindle tumor cells from irradiated mice (Goldfeder, 1965). Changes following 200 to 400 rads of X-irradiation included swelling of mitochondria, loss of mitochondrial cristae, dilation of endoplasmic reticulum, breakage of plasma membranes, and aggregations of RNP particles. Increases in ATPase and catalase activity suggested changes in permeability of boundary membranes. It was suggested that radioresistant cells had more organelles, such as mitochondria, that were not entirely destroyed or were able to function and reproduce. Jordan (1972) described early mitochondrial changes in mice irradiated with 600,000 rads of 8.5 mev X rays. These changes included: mitochondrial swelling, partial dissolution of mitochondrial cristae, and homogeneous intramatrical densities.

Studies on lymph nodes from X-irradiated rats showed a difference in sensitivity of cells (Holsten, 1970). Lymphocytes were the most sensitive to irradiation, followed by other cells of the lymphocytic series and differentiated reticulum cells. Plasma cells were described as the most radioresistant of the cells in the lymph nodes. Other studies on

the lymph nodes of X-irradiated mice described histiocytes forming prominent pseudopodia within 30 minutes after irradiation with 400 roentgens of x rays (Smith, et al., 1967), and histiocytes containing many phagocytized necrotic lymphocytes after 6 hours.

The injection of colloidal gold (Au^{198}) in lymphatic vessels has been used therapeutically in man for treating metastatic neoplasms in lymph nodes. Rather marked histopathologic changes were observed in lymph nodes from dogs injected with 2 mc to 85 mc of Au^{198} (Christopherson and Berg, 1955). Changes at 3 days consisted of focal areas with sinusoids filled with mononuclear cells, cytoplasmic vacuolation in reticular cells, and occasional giant cells with cytoplasmic vacuoles. Areas of demonstrable radioactivity had depleted lymphoid tissues with disrupted germinal centers, atypical mitotic figures, hyperchromatic-to-pyknotic nuclei, plasma cells, thrombi, and necrotic debris. At 3 to 14 days there was focal necrosis in cortical or medullary areas and fibrinoid necrosis of blood vessels. Scar tissue was pronounced after 5 to 6 weeks. A decreased filtration capacity for colloidal gold Au^{198} occurs in x-irradiated lymph nodes (Sinha and Goldenberg, 1970).

Cable (1962) described lymphoid necrosis, reticuloendothelial cells filled with debris, and a heavy infiltration of granulocytes in prefemoral lymph nodes from a pig injected intradermally with plutonium nitrate. A total of 0.3 microcuries of Pu-239/gram of lymph node (wet weight) accumulated after 168 hours. Autoradiographic

activity in these lymph nodes was associated with macrophages and reticuloendothelial cells of lymph sinuses. The late effect of intradermal administration of plutonium in swine included hyperplasia in germinal centers of systemic lymph nodes and a lymphomatous hepatic node (McClanahan, 1968).

The tracheobronchial lymph nodes of dogs used in plutonium inhalation studies have numerous changes (Park, et al., 1962; Park, et al., 1964; Clark, et al., 1966). With increasing time and dose, these changes have included loss of lymphocytes, fibroplasia, unmasking of reticular cells, and accumulation of macrophages filled with hemosiderin. In the final stages the tracheobronchial lymph nodes were composed of dense sclerotic connective tissue devoid of any lymphatic elements.

The superficial cervical lymph nodes of dogs injected with plutonium nitrate in the forepaw had local histopathologic changes that were described (Watters, et al., 1971). These changes included necrosis, reticular cell hyperplasia, fibrosis, and occasionally granulomatous inflammation that extended into the perinodal tissues. In this study, the severity of change was related to the dose administered. Lower doses of plutonium were concentrated in the superficial cervical lymph nodes without any histopathologic changes.

Electron microscopic studies have not been reported on lymph nodes containing plutonium particles, but the ultrastructure of phagocytes in the peritoneal cavity of rats and alveolar macrophages in

rabbits have been studied after plutonium injection. Sanders and Adee (1970) used electron microscopic autoradiography to localize the plutonium in phagocytes from the peritoneal cavity of rats. At 2 to 6 hours after plutonium administration there was increased cytoplasmic vacuolization. These vacuoles contained engulfed plutonium particles and unidentified cell debris. Lysosomal-like structures seemed to increase in number. At 1 day after plutonium administration there were increased numbers of pseudopodial projections, increase in size of phagocytes, dilated Golgi cisternae, dispersed endoplasmic reticulum, increased amounts of cell debris in vacuoles, and some engulfed degenerating lymphocytes. Changes in phagocytes at 7 days after plutonium administration included increased amounts of membranous laminated myelin-like structures, lysosomal-like structures containing particles resembling plutonium, numerous psudopodia, marked swelling, many phagocytized cells, and large vacuoles. The cytoplasm of lymphocytes at 7 days was swollen and contained partially digested mitochondria, dilated endoplasmic reticulum and Golgi apparatus, while in the nucleus chromatin clumping and lysis were observed. The phagocytized plutonium particles appeared to be causing extensive destruction of lymphocytes.

The ultrastructural changes in alveolar macrophages from rabbits exposed to plutonium oxide particles have been studied. Lutz, et al., (1970) considered that the alveolar macrophages were able to break up larger particles to present agglomerations of granules. The

alveolar macrophages had distinct halos around the plutonium particles and an abundance of microfilaments in their cytoplasm. The process of phagocytosis of plutonium particles by alveolar macrophages obtained from pulmonary lavages of rabbits was demonstrated with microcinematographs by Nolibe (1973).

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

A total of 19 adult male beagles was obtained from the barrier maintained beagle colony of the Collaborative Radiological Health Laboratory at Colorado State University. The beagles were housed outdoors at the Foothills Research Campus of Colorado State University in kennels designed by the Collaborative Radiological Health Laboratory. The beagles were fed ad libitum with dry Purina dog chow* and watered with pans or an automatic watering system. The beagles were observed daily and regular veterinary medical care was provided, including vaccination for rabies, canine distemper, and infectious canine hepatitis.

Plutonium Oxide

The high fired plutonium oxide was supplied by the Rocky Flats Division of the Dow Chemical Company in 5 ml pharmaceutical ampules fitted with airtight rubber diaphragms. Each vial contained approximately 1 mg. The high fired oxide was prepared from electrorefined unalloyed plutonium metal dissolved in HCl and precipitated with oxalic acid. The

*Lab Dog Chow, Ralston Purina Co., St. Louis, Mo.

washed salt was calcined to the high fired state of plutonium dioxide for 100 hours at 850 degrees Celsius, dried at 80 degrees Celsius for approximately 100 hours, and pulverized by grinding in a tungsten carbide grinding vial. The stoichiometry of the oxide formed was accepted as $PuO_{1.98}$. The particle size was measured by optical microscopy. The physical, chemical, and isotopic properties of the oxide are listed in Tables 2 and 3.

The amount of plutonium in lymph nodes at necropsy was determined by extraction and liquid scintillation procedures similar to those described by Keough and Powers (1970). The lymph node specimens were reduced to carbon-free ash in new 100 ml beakers by digestion with hot concentrated nitric acid and muffling at 450 degrees Celsius. The ash was soaked with 6 M nitric-6 M hydrofluoric acid and twice evaporated to dryness. Then the residue was evaporated to dryness in 8 M nitric acid and dissolved in hot 2 M nitric acid containing 0.2 M boric acid to make a final solution containing 1 to 15% dissolved salts. A 0.1 ml aliquot of this solution was added to 10 ml of 2 M nitric acid, and a drop of 4 M urea, in 20 ml counting vials. The extractant-scintillator solution (4 ml) was added, the vials shaken vigorously for 30 seconds, and the shaking repeated 5 times at 5 minute intervals. The alpha activity in the vials was then determined by liquid scintillation. The disintegrations per minute were multiplied by 100 to compensate for the dilution made with 2 M nitric acid.

Table 2. Chemical and Isotopic Properties of Plutonium

Pu	0.8826 g/g PuO_2
Cation impurities	≈200 ppm (based on Pu)
^{241}Am	360 ppm based on Pu as of Oct. 1, 1970
^{241}Am growth rate	≈15 ppm/month based on Pu
^{238}Pu	0.0105 wt %, based on Pu
^{239}Pu	93.898 wt %, based on Pu
^{240}Pu	5.695 wt %, based on Pu
^{241}Pu	0.377 wt %, based on Pu as of June, 1970
^{242}Pu	0.019 wt %, based on Pu
Atomic wt of Pu	239.116 as of June, 1970

Table 3. Physical Properties of Plutonium

Particle Size	
1μ or less	68%
1μ to 3μ	21%
3μ to 5μ	4%
5μ to 10μ	6%
>10μ	<1%

Mass Median Diameter Approximates .7μ

Experimental Procedures

The beagles were assigned to experimental groups (Table 4) and were brought indoors for all experimental procedures. The laboratory was prepared for the injection of plutonium by covering the walls and floors with plastic and waxed paper. During the implanting procedures, personnel-protective equipment, including respirators, was used and surveillance was made by radiation monitoring personnel from the health physics group of the Rocky Flats Division of the Dow Chemical Company. The dogs were kept in metabolism cages to collect urine and feces for a few days after implantation; these wastes were delivered to the Rocky Flats installation for disposal.

The plutonium oxide was injected subcutaneously into the dorsal surface of the left hind paw of 15 dogs (Groups I through V), and the right hind paw of one dog from each of Groups II through V. The dogs were given a preanesthetic injection of propiopromazine hydrochloride* and anesthetized with intravenously administered pentobarbital sodium. The injection site was clipped of hair, cleaned with 70% ethyl alcohol, and isolated with paper towels. The insoluble plutonium oxide particles in 5 ml vials were suspended in 1.0 ml of physiological saline solution and 0.25 ml of this suspension was withdrawn into a 1 ml tuberculin glass syringe. The glass syringe was attached to a 21 gauge needle pre-positioned subcutaneously. Proper positioning of the needle was

*Tranvet, Diamond Laboratories, Desmoines, Iowa.

Table 4. Experimental Design: Utilization of Dogs

Group & Dog No.	Inject PuO_2 Rear Paw		Amputate Tarsus	DTPA	Necropsy
	Left	Right			
Group I					
2398	yes	no	no	no	4 weeks
2515	yes	no	no	no	"
2546	yes	no	no	no	"
Group II					
2396	yes	no	4th week	no	8 weeks
2509	yes	no	" "	no	"
2405	yes	yes	" "	no	"
Group III					
2426	yes	no	" "	no	16 weeks
2536	yes	no	" "	no	"
2386	yes	yes	" "	no	"
Group IV					
2403	yes	no	" "	no	32 weeks
2535	yes	no	" "	no	"
2527	yes	yes	" "	no	"
Group V					
2406	yes	no	" "	yes	32 weeks
2526	yes	no	" "	yes	"
2397	yes	yes	" "	yes	"
Controls:					
2540	no	no	" "	no	8 weeks
2519	no	no	" "	no	16 weeks
2541	no	no	" "	no	32 weeks
2534	no	no	" "	yes	32 weeks

determined by aspiration to ascertain that no back flow of blood occurred that would indicate the accidental intravenous injection of plutonium. The plutonium was then injected into the subcutaneous tissues.

A calibration technique was devised (Bistline, 1972) to determine the amount of plutonium implanted into each dog. A Germanium (Li) detector was used to measure the amount of material contained in each vial prior to implanting 2 dogs from each vial. Following implantation, the activity was measured in the injected paws and what remained in the vial. A ratio of activity in the paws could then be correlated to an accurate measurement of the amount used from each vial.

The injected paws, and corresponding popliteal lymph nodes, were monitored in situ approximately twice a week for radioactivity with a NaI wound counter. The wound counter, a 2-inch diameter by 4 mm thick NaI (Tl) scintillation detector with a thin beryllium window, measured 17 kev L X rays of uranium and neptunium, which follow the decay of plutonium and americium. The wound counter data were calibrated with a source having a similar ratio of uranium and neptunium L X rays as the injected material.

The amount of plutonium in the popliteal lymph nodes during the antemortem phase of the study was calculated from the counts obtained with the wound counter. Since the efficiency of the wound counter varied during the course of the study, the counts per minute were

normalized to 1000 counts per minute obtained with a 1 microcurie source of plutonium. The normalized counts on the day of necropsy were then related to the disintegrations per minute found in the lymph node with liquid scintillation procedures. The conversion factor obtained on the day of necropsy was used retrospectively to estimate the amount of plutonium in the lymph node from the day of implantation. Regression curves were determined with a Hewlett-Packard Calculator* using a polynomial regression program.

Approximately 4 weeks after implantation of the plutonium, the left hind leg was amputated at the tarsus for the dogs in Groups II through V and for the control groups. Each dog was given a preanesthetic injection of propiopromazine hydrocholride and anesthetized with an intravenous injection of pentobarbital sodium. The hair from the left hind leg was clipped and the leg was washed with soap and water. The surgical site was cleaned with 70% ethyl alcohol and isolated with sterile drapes. A skin incision was made around the circumference of the leg about 2 cm below the tarsus and extended proximally to the tarsus on the anterior and posterior surfaces. The skin was reflected proximally and a tourniquet was tightened around the leg to stop circulation. A scalpel was then used to amputate through the tarsus at a location chosen to leave the tibial and fibular tarsal bones intact. The tourniquet was released and the major arteries ligated with chromatic 00 catgut. The skin was trimmed with scissors

*Model 9810, Hewlett-Packard, 815 14 S. W., Loveland, Colo.

to fit the stump and sutured with No. 1 silk. Bandages were applied and changed daily for several days. Each dog received 2 cc of a penicillin and streptomycin mixture daily, injected into muscles of the forelimb, for 5 days. Skin sutures were removed in 10 to 14 days.

One dog in Group II (No. 2398) did not recover from deep anesthesia following surgery and the next day was shifted into Group I and killed. Another dog originally intended for Group I was subsequently transferred into Group II as a replacement. The remaining dogs recovered promptly from the amputation surgery and rapidly became accustomed to walking on 3 legs. After a few weeks there seemed to be very little diminution of motility.

The trisodium calcium salt of diethylenetriaminepentaacetic acid (DTPA) was administered intravenously to dogs in Group V and to one control dog. Injections of 0.25 gm of DTPA dissolved in 10 ml of physiological saline were made into the jugular vein twice weekly from the time of amputation to necropsy.

Necropsy and Histopathology

At the time of sacrifice the dogs were anesthetized with pentobarbital sodium and the following lymph nodes were bilaterally removed and fixed in 10% buffered formalin: popliteal, external iliac, superficial inguinal and deep inguinal (when present). These lymph nodes were later weighed and monitored for radioactivity with a NaI wound counter. After the lymph nodes were removed, the dogs were

killed by exsanguination. The carcasses were frozen for later disposal supervised by the Radiation Health Officer at Colorado State University.

Specimens for histopathologic evaluation were routinely processed, embedded in paraffin, cut at 6 microns, and stained with hematoxylin and eosin. Each dog had 2 sections prepared from different areas of the left and right popliteal lymph nodes, and 1 section prepared from each left and right external iliac lymph node. In addition, Dog 2397 (Group V) had sections prepared from the superficial inguinal lymph nodes. Special stains applied to selected slides were Prussian blue for iron and Masson's trichrome for connective tissues.

Autoradiographs were prepared by dipping paraffin sections of lymph nodes in Ilford K-5* emulsion and exposing the sections for 24 hours before developing with Dektol.** Similar autoradiographs exposed for 1 hour were prepared from contaminated popliteal lymph nodes, randomly sorted, and coded to hide their identity. Six random fields from the 1 hour exposed autoradiographs were selected for examination with a 40X objective on a Zeiss*** microscope (8X eyepieces and 2X Optovar). In each field the total number of alpha track sources and the total number of tracks per source were tabulated. Since it was usually not possible to distinguish the point source of an

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*Ilford Nuclear Research Emulsion, Ilford Limited, Ilford, Essex.
**Eastman Kodak Co., Rochester, N. Y.
***Carl Zeiss, 7082 Oberkochen, West Germany.

alpha star, a source was considered to approximate the size of a cell 8 microns in diameter.

Electron Microscopy

Specimens for electron microscopy, selected from the left and right popliteal and external iliac lymph nodes, were immediately immersed in cold 2% gluteraldehyde buffered in Tyrode's solution. After 4 hours fixation the specimens were washed and held in cold Tyrode's buffer overnight, post-fixed in osmium tetroxide, processed in a routine manner, and embedded in epoxy resin (Epon 812*). A total of 10 to 20 random blocks were prepared from each lymph node.

Autoradiographs were prepared from 1 micron thick Epon sections of popliteal lymph nodes that were cut with glass knives on a Porter-Blum** ultramicrotome. These sections were dipped in Ilford K-5 emulsion, held in refrigerated light-tight boxes for 1 to 3 days, developed with Dektol, and examined for autoradiographic localization of plutonium particles.

Thin sections, cut at approximately 600 Angstroms thick with a diamond knife on an LKB*** Ultratome, were prepared from blocks shown by autoradiography to contain plutonium particles. The thin sections were mounted on uncoated 150 or 300 mesh copper grids, stained with uranyl acetate and lead citrate, and examined with 50 KV

*Ladd Research Industries, Inc., Burlington, Vt.
**Sorvall MT-2, Ivan Sorvall, Inc., Norwalk, Conn.
***LKB Instruments Inc., Rockville, Md.

on an RCA EMU-3G* electron microscope located at the Collaborative Radiological Health Laboratory, Colorado State University. Additional specimens were examined with 100 KV on an RCA EMU-4* electron microscope at Battelle Pacific Northwest Laboratories.

Electron microscopic autoradiography was performed on one specimen from the left popliteal lymph node of Dog 2536 from Group III (16 weeks) that had alpha activity with light microscopy. The loop method (Caro, 1962) was used with Ilford L-4 emulsion on collodion coated copper grids. The grids were developed with D-19** after 2 weeks exposure.

Electron diffraction patterns of plutonium particles were obtained from popliteal lymph node specimens examined from Dog 2386 with a Phillips 200*** electron microscope at Rocky Flats, Colorado. Bright field pictures, and selected area diffraction patterns of particles were taken at 100 KV accelerating voltage. An aluminum standard was used to calculate the camera constant used for indexing the diffraction patterns ($d = \frac{L\lambda}{r}$; where d = spacing in crystal, L = camera constant, and r = radius).[1] Electron diffraction patterns from plutonium particles were also searched for on a JOEL**** 1 megavolt electron microscope at the University of Colorado in Boulder; no diffraction patterns were obtained with this scope.

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[1]ASTM Powder Diffraction File Card No. 6-360 (PuO_2) was used as a diffraction reference.

*Radio Corporation of America, Camden, New Jersey.

**Eastman Kodak Co., Rochester, N. Y.

***N. V. Phillips, Eindhoven, Netherlands.

****Japan Electron Optics Laboratory, Akishima, Tokyo, Japan.

CHAPTER IV

RESULTS

Plutonium Analysis

The percent of the implanted dose recovered in the popliteal lymph node specimens at necropsy ranged from 1% to 12% with a mean of 5% (Table 5). It is noteworthy that the right popliteal lymph node, which drained plutonium injection sites for a longer period of time, contained a higher mean percent of the implant dose than did each of the contralateral left popliteal lymph nodes from amputated sides.

The external iliac lymph nodes showed a significant amount of plutonium that drained from the popliteal lymph node in several dogs (Table 6). The amount of plutonium present, however, was generally small compared to the amount present in the popliteal lymph node.

External (in situ) Counting Data

The radioactivity of paws injected with plutonium, as measured by an external wound counter, decreased for approximately 3 to 4 weeks (Fig. 1). The radioactivity seemed to stabilize at that time, with the paws retaining about 70% of their initial implant radioactivity.

Table 5. Radiochemical Analysis: Plutonium in Paw and Popliteal Lymph Nodes

Group & Dog No.	Implant left paw μ Ci	Left popliteal lymph node μ Ci	(% implant)	right paw μ Ci	Right popliteal lymph node μ Ci	(% implant)
Group I (4 weeks)						
2398	15.6	1.0	(6)			
2515	39.4	1.0	(3)			
2546	14.6	0.8	(5)			
Group II (8 weeks)						
2396	11.9	0.3	(2)			
2509	21.0	1.4	(7)			
2405	22.6	0.4	(2)	9.2	0.5	(5)
Group III (16 weeks)						
2426	27.5	0.8	(3)			
2536	16.9	0.7	(4)			
2386	18.8	0.4	(2)	15.6	0.7	(4)
Group IV (32 weeks)						
2403	19.1	0.3	(2)			
2535	24.3	2.0	(8)			
2527	19.2	0.5	(3)	13.9	1.6	(12)
Group V (32 weeks)						
2406	24.0	0.2	(1)			
2526	10.6	1.1	(10)			
2397	36.0	2.9	(8)	17.7	1.6	(9)

Table 6. Radiochemical Analysis: Plutonium in External Iliac and Superficial Inguinal Lymph Nodes

Group & Dog No.	Left External Iliac Lymph Node dpm/gram	Right External Iliac Lymph Node dpm/gram	Left Superficial Inguinal Lymph Node dpm/gram	Right Superficial Inguinal Lymph Node dpm/gram
Group I (4 weeks)				
2398	758	435		
2515	873	662		
2546	4, 682	N. S.		
Group II (8 weeks)				
2396	343	496		
2509	13, 466	700		
2405*	1, 209	6, 054		
Group III (16 weeks)				
2426	4, 683	1, 261		
2536	566, 642			
2386*	404, 312			
Group IV (32 weeks)				
2403	796	502		
2535	7, 055	608		
2527*	59, 010	52, 420		
Group V (32 weeks)				
2406	44, 177	174		
2526	6, 894	291		
2397*	15, 424	2, 764, 678	1, 845	403, 134

*Plutonium was injected into the left and right paw.
N. S. Not significantly greater than background.

Plutonium rapidly reached the popliteal lymph nodes. In several dogs plutonium was detected with the wound counter a few minutes after injection. After about 4 weeks, when the level of radioactivity detectable in the popliteal lymph nodes with the wound counter had stabilized, the left hind paw was amputated. The day of amputation was considered as "day 1" for purposes of determining clearance of plutonium from popliteal lymph nodes, since at this time there could be no continued movement of plutonium to the popliteal lymph node from the amputated paw.

The in situ wound counting data showed no clearance of plutonium from the popliteal lymph nodes from dogs in Group II (Fig. 2, Table 7). Dog 2405 (Group II), however, had a greater increase in radioactivity in the right popliteal lymph node, draining the non-amputated right paw injected with plutonium, than occurred in the left popliteal lymph node, draining the amputated left paw injected with plutonium. The data from Group III dogs showed a slight clearance of plutonium from the left popliteal lymph node, as evidenced by a negative linear regression slope in 1 dog (No. 2426), but positive linear regression slopes in the other 2 dogs (Fig. 3, Table 7) indicating that there was no clearance of plutonium. There was no significant difference between the left and right popliteal lymph node regression slopes of dog 2386 (Group III) injected with plutonium in both the left and right hind paws.

Table 7. Lymph Node Clearance Curves on Dogs from Groups II through V from 1 Day after Amputation

Group, Dog Number, & Lymph Node*	Y Intercept	Slope
Group II		
2396L	0.031	+0.000047
2509L	0.067	+0.000041
2405L	0.015	+0.0000030
2405R	0.040	+0.00031
Group III		
2426L	0.028	-0.000027
2536L	0.033	+0.000040
2386L	0.016	+0.000042
2386R	0.043	+0.000022
Group IV		
2403L	0.027	-0.0000013
2535L	0.085	-0.000041
2527L	0.026	+0.000024
2527R	0.12	+0.000039
Group V		
2406L	0.006	+0.0000099
2526L	0.107	+0.000032
2397L	0.087	+0.000011
2397R	0.152	-0.00033

* L = Left Popliteal lumph node
R = Right Popliteal lymph node

The left popliteal lymph node external counting data from dogs in Groups IV and V (Figures 4 and 5, Tables 7 and 8) was plotted from day 100 to provide information on plutonium concentration after dogs in Groups II and III were sacrificed. No significant differences were found (using Student's t test) between the linear regression coefficients of the left popliteal lymph nodes from the dogs in Groups IV (no DTPA) and V (DTPA). The slopes were negative in 5 out of 6 dogs, and the slope was essentially flat in the remaining dog (No. 2527).

The right popliteal lymph node external counting data showed positive regression slopes (Fig. 6, Table 7) in each dog except dog 2397. The right popliteal lymph node of dog 2397 had markedly reduced activity with the wound counter for several days at approximately 3 months and again 5 months after amputation. At these times perinodal tissues became firm, enlarged, and warm. The decreased counts were clearly due, in part, to increased tissue absorption of the 17 kev X rays. During these times the dog was febrile and was treated with penicillin. Several syringes of brownish fluid were aspirated from a fluctuant subcutaneous area over the right popliteal lymph node; over 100 counts per 40 seconds were counted in one of these syringes containing brown fluid. After necropsy, plutonium was detected in the superficial inguinal lymph nodes with the wound counter, further suggesting some spillage into the extranodal tissues of the leg. This was the only animal with detectable amounts of wound counter activity in the superficial inguinal lymph nodes.

Table 8. Lymph Node Clearance Curves on Dogs from Groups IV and V from 100 Days after Amputation

Group, Dog Number, & Lumph Node*	Y Intercept	Slope
Group IV		
2403L	0.045	-0.00012
2535L	0.116	-0.00024
2527L	0.028	+0.0000092
Group V		
2406L	0.011	-0.000017
2526L	0.116	-0.000018
2397L	0.134	-0.00029

* L = Left Popliteal lymph node

Weights of Popliteal Lymph Nodes

The weights of the popliteal lymph nodes are tabulated in Table 9. Since the weights of the popliteal lymph nodes were quite variable among dogs, it seems appropriate to compare the right and left popliteal lymph nodes in individual dogs. In each control dog having the left hind paw amputated longer than four weeks before necropsy, the left popliteal lymph node was lighter than the right popliteal lymph node.

The earlier effects of plutonium, observed in dogs from Group I, tended to increase lymph node weights. At later time intervals, popliteal lymph nodes having plutonium generally weighed less than contralateral popliteal lymph nodes not containing plutonium. It must be pointed out also that the weights recorded were actually more scar tissue than functioning lymph node parenchyma.

Histopathology of Popliteal Lymph Nodes (Tables 10 & 11)

The right popliteal lymph nodes in the control dogs were not significantly different in morphology from the right popliteal lymph nodes in groups of dogs injected with plutonium in the left paw. The cortex was composed of lymphoid nodules with variable sized germinal centers. The germinal centers generally had a crescent of small lymphocytes on the capsular side. Scattered foci of phagocytosed nuclear debris were present in germinal centers in direct proportion

Table 9. Lymph Node Weights at Necropsy

Group & Dog Number	Left Popliteal Lymph Node, g.	Right Popliteal Lymph Node, g.
Group I (4 weeks)		
2398	1.07	0.56
2515	0.55	0.48
2546	0.48	0.43
Group II (8 weeks)		
2396	0.73	0.58
2509	0.55	0.63
2405	1.08	0.89
Group III (16 weeks)		
2426	0.54	0.48
2536	0.28	0.63
2386	0.46	0.42
Group IV (32 weeks)		
2403	1.11	1.28
2535	0.56	0.78
2527	0.43	0.32
Group V (32 weeks)		
2406	0.89	0.88
2526	0.34	0.55
2397	0.65	0.44
Controls:		
2540 (8 weeks)	0.53	0.49
2519 (16 weeks)	0.28	0.48
2541 (32 weeks)	0.41	0.84
2534 (32 weeks)	0.44	1.63

Table 10. Histopathologic examination of left popliteal lymph node.

Dosage Group & Animal Number	Inflammatory Changes									Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis	cavitations	autoradiographic activity	number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																							
2398	±	2	1	2	1	0	0	0	3	2	2	0	2	2	1	1	1	1	1	±	2	2	1
2515	±	2	2	2	1	±	0	0	3	1	±		1	2	1	1	0	2	1	1	±	±	1
2546	1	2	1	2	1	±	0	0	3	2	1	0	2	2	1	1	±	3	1	1	0	0	1
Group II (8 weeks)																							
2396	1	1	1	1	1	0	0	0	3	1-2	2	0	2	2	1	±	1	2	1	±	±	0	1
2509	3	1	±	1	2	2	2	0	3	1	0	0	1	1	1	±	±	2	1	±	0	0	±
2405	2	2	1	1	1	0	0	0	3	1-2	2	0	2	2	1	±	1	1	1	±	±	±	±
Group III (16 weeks)																							
2426	2-3	1	1	1	1-2	1	2	0	3	2	2	0	2	2	1	1	1	2	1	1	1	1	1
2536	2	1-2	±	1	1	±	1	0	3	1	1	0	2	2	1	1	1	3	1	±	±	±	±
2386	2	2	1	2	1	±	1	0	3	1	2	0	2	1	1	1	1	2	1	1	1	1	1
Group IV (32 weeks)																							
2403	1	1	1	1	1	1	0	0	2	1	1	0	2	2	1	1	±	2	1	1	1	0	1
2535	3	2	1	1	2	±	1	0	3	1	2	0	2	2	1	±	1	2	1	1	±	0	1
2527	3	1	1	2	1	1	2	0	3	1	1	0	2	2	1	1	1	2	1	1	1	0	1
Group V (32 weeks)																							
2406	3	2	1	1	1	±	0	0	3	1	±	0	0	0	0	0	0	0	0	0	0	0	0
2526	3	1	1	2	1	±	2	0	3	1	±	0	2	2	±	1	1	2	±	1	1	0	±
2397	3	2	1	2	2	1	2	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls (8 weeks)																							
2540	0	0	0	0	0	0	0	0	0	2	2	1	1-2	1	1	±	1	2	1	0	±	±	0
(16 weeks)																							
2519	0	0	0	0	0	0	0	0	0	1	1	2	1	±	1	0	2	2	1	0	0	0	0
(32 weeks)																							
2541	0	0	0	0	0	0	0	0	0	2	1	±	1	1	1	±	±	2	1	±	±	±	0
(32 weeks)																							
2534	0	0	0	0	0	0	0	0	0	2	2	±	2	2	1	±	±	1	1	±	±	±	±

Key =
0=none
0+=trace
±=very slight (or very small amount)
±-1=very slight-to-slight (or very small-to-small amount)
1=slight (or small amount)
1-2= slight-to-moderate (or small-to-moderate amount)
2=moderate
2-3=moderate-to-marked
3-4=marked-to-extreme
4=extreme

Table 11. Histopathologic examination of right popliteal lymph node.

Dosage Group & Animal Number	Inflammatory Changes								Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis	autoradiographic activity	number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																						
2398	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	2	1	±	1	2	1
2515	0	0	0	0	0	0	0	0	2	2	1	2	2	1	0	±	1	1	±	0	0	±
2546	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	±	2	1	1	1	±	1
Group II (8 weeks)																						
2396	0	0	0	0	0	0	0	0+	2	2	0	1	1	1	±	0	2	1	±	±	0	0
2509	0	0	0	0	0	0	0	0	2	2	±	1	1	1	1	0	3	1	±	±	0	0
2405	2	3	1	1	1	0	0	3	1	1	0	2	2	1	±	±	2	1	±	±	1	1
Group III (16 weeks)																						
2426	0	0	0	0	0	0	0	0	2	1-2	0	1-2	2	1	1	1	2	1	1	±	1	1
2536	0	0	0	0	0	0	0	0+	2-3	2	±	2	2	1	±	0	2	1	±	0	±	±
2386	4	0	1	2	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group IV (32 weeks)																						
2403	0	0	0	0	0	0	0	0	2	2	0	2	2	1	1	0	2	1	±	±	0	0
2535	0	0	0	0	0	0	0	0	2	2	±	2	2	1	±	1	2	1	±	±	±	1
2527	2	2	1	1	1	±		2-3	1	2	0	1	1	1	1	±	2	1	1	1	1	1
Group V (32 weeks)																						
2406	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	±	1	1	±	±	±	±
2526	0	0	0	0	0	0	0	0	2	2	0	2	2	1	±	0	2	1	±	0	0	0
2397	3-4	1	1	2	2	±	2	3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls (8 weeks)																						
2540	0	0	0	0	0	0	0	0	2	2	1	1-2	1	1	±	1	2	1	0	±	±	0
(16 weeks)																						
2519	0	0	0	0	0	0	0	0	2	2	0	2	1	1	1	±	2-3	1	1	±	±	0
(32 weeks)																						
2541	0	0	0	0	0	0	0	0	2	1	±	2	2	1	1	±	2	1	1	±	±	1
(32 weeks)																						
2534	0	0	0	0	0	0	0	0	3	2-3	0	2-3	2	1	±	1	1	1	±	1	±	1

Key =
0 = none
0+ = trace
± = very slight (or very small amount)
±-1 = very slight-to-slight (or very small-to-small amount)
1 = slight (or small amount)
1-2 = slight-to-moderate (or small-to-moderate amount)
2 = moderate
2-3 = moderate-to-marked
3-4 = marked-to-extreme
4 = extreme

to the size of the germinal centers. Foci of adipose tissue were occasionally seen between the nodules in various areas of the cortex. The paracortical areas consisted of a rather uniform population of lymphocytes that blended into the medullary cords which varied in thickness and were composed of plasma cells, lymphocytes, and neutrophils on a supporting framework of reticular cells. The medullary cords had scattered fragments of nuclear debris, and Russell bodies that were present in direct proportion to the number of plasma cells. The medullary sinuses had numerous diffusely distributed macrophages and lymphocytes. Areas of hemorrhage, erythrophagocytosis, and neutrophilic infiltration were common in the medullary sinuses. Eosinophils, mast cells, and magakaryocytes were occasionally present. Small amounts of hemosiderin were common in both the medullary cords and medullary sinuses. The cellular content of the subcapsular sinuses generally reflected the content of the medullary sinuses.

The effects of amputation on popliteal lymph node morphology were observed in the control dogs. Aside from dog 2540, in which the left paw was amputated 4 weeks before necropsy, the general trend in morphology of the left popliteal lymph node after amputation was atrophy. The left popliteal lymph node from dog 2519, removed approximately 3 months after amputation, had fewer cortical nodules, smaller germinal centers, thinner medullary cords, and fewer cells in the medullary sinuses than the contralateral right popliteal lymph

node. Seven months after amputation, the left popliteal lymph node from dog 2541 had thinner medullary cords with reduced numbers of plasma cells, and the left popliteal lymph node from dog 2534 had fewer cortical nodules, smaller germinal centers, and thinner medullary cords.

The effects of plutonium were present in the left popliteal lymph nodes from dogs in Group I (4 weeks). Moderate sized, irregularly shaped areas of cortex were replaced by increased numbers of reticular cells mixed with macrophages, fibrocytic proliferation, a few collagen fibers, and diffuse infiltration with neutrophils. The mixture of inflammatory cells extended in areas through the capsule, generally obscured the subcapsular sinus, and blended with remnants of lymphoid tissue in the cortex and medulla. The areas replaced by inflammation had moderate numbers of pyknotic and karyorrhexic nuclei diffusely distributed among the reticular cells and adjacent lymphoid tissues (Figures 7 and 8), small amounts of hemosiderin phagocytized by macrophages, and very small amounts of hemorrhage.

Inflammatory changes occupied relatively large areas of the left popliteal lymph nodes of dogs in Group I (4 weeks), but the normal morphology was intact in most areas of these lymph nodes. Although some cortical nodules were largely replaced with reticular cells, other normal appearing nodules had germinal centers. The medullary sinuses had marked numbers of macrophages in one dog, which was probably a reflection of the inflammatory reaction in other

areas of the node. A moderate amount of hemorrhage in the medullary sinuses of dog 2398 was probably from the amputation surgery the previous day; this dog was not originally intended for Group I, but was necropsied since it never recovered from anesthesia.

Small amounts of scar tissue replaced areas of cortex in the left popliteal lymph nodes from dog 2396 (Group II) (Fig. 9) and dog 2403 (Group IV) injected with plutonium into the left paw. The scar tissue consisted primarily of fibroblasts and collagen mixed with small numbers of reticular cells, macrophages, neutrophils, necrotic debris, and, in dog 2403, hemorrhage. Although the cellular infiltrate extended into the medulla and the capsule, the general morphology of these lymph nodes remained intact with large areas of essentially normal cortex and medulla.

The right popliteal lymph node from dog 2405 (Group II) had large areas of cortex replaced by reticular cells that were diffusely mixed with fibroplasia, macrophages, neutrophils, scattered necrotic debris, and hemosiderin. The cellular infiltration extended into the capsule. The cortex still had small numbers of remaining lymphoid nodules with small germinal centers. The medulla was relatively normal.

Moderate to extreme amounts of scar tissue (Fig. 10) replaced large areas of the popliteal lymph nodes draining plutonium injection sites in the remaining dogs from Groups II (8 weeks) through V (32 weeks). The scar tissue in these lymph nodes was generally

composed of dense fibrous tissue encapsulating areas up to 0.5 cm in diameter composed of strands of collagen in an eosinophilic matrix that became progressively more acellular toward the center. The eosinophilic matrix contained a few fibroblasts and collagen fibers, a few widely scattered neutrophils and macrophages, a small amount of diffusely distributed pyknotic and karyorrhexic debris, and hemosiderin (Figs. 11 and 12). The hemosiderin was generally phagocytosed by macrophages but toward the center of the scar tissue often appeared extracellular. In several lymph nodes from dogs in Groups II through V, moderate sized foci of necrotic debris were mixed with the golden brown pigment. Foci of hemorrhage were frequently present. Medium sized muscular arteries in the center of the scar tissue frequently had smudgy, necrotic walls. Small areas of cavitation (Fig. 13) were present in the left popliteal lymph node of dog 2397; these cavities appeared largely void of contents and were lined with scar tissue.

Adjacent to the scar tissue, most of the heavily involved popliteal lymph nodes had increased numbers of reticular cells and macrophages replacing further areas of the cortex and medulla. Occasional mitotic figures were present among the reticular cells. Small foci of plasma cells and lymphocytes were frequently in the capsule or surrounding perinodal tissue. The popliteal lymph nodes from several dogs in Groups II through V had no recognizable cortex and/or medulla.

The autoradiographs showed the distribution of plutonium particles was related to lymph flow through the popliteal lymph node. In the dogs from Group I the plutonium particles were found primarily in the subcapsular areas and the areas between the cortical nodules corresponding to Zone 1 of Nopajaroonsri (1971). Although the morphology of the lymph nodes was severely altered, in the groups of dogs exposed to plutonium for longer periods of time, the plutonium particles were more concentrated in the medullary areas nearer the efferent lymphatics. The lymph flow was probably severely altered, however, by the formation of scar tissue.

Autoradiographs of the left popliteal lymph nodes exposed for 24 hours had marked numbers of large alpha stars and alpha clusters aggregated in areas of scar tissue and necrosis (Figs. 14 and 15). The alpha activity was more pronounced over regions where hemosiderin was deposited (Figs. 16 and 17), but alpha tracks occasionally originated from macrophages lacking any discernible pigment (Figs. 18 and 19). There was scattered alpha activity in areas with increased numbers of reticular cells. The right popliteal lymph nodes from dogs injected with plutonium into the right paw had similar amounts of alpha activity, although the right popliteal lymph node of dog 2397 had overlapping large alpha stars denser than any other section. Traces of alpha activity, composed of single tracks, were present in several popliteal lymph nodes not draining injection sites;

alpha stars, however, were only present in lymph nodes draining injection sites.

In autoradiographs exposed for 1 hour, alpha tracks counted from different random microscopic fields (Fig. 20, Table 12) showed that the average numbers of tracks per plutonium source increased as a function of time. There were other very concentrated areas of alpha activity in the popliteal lymph nodes of dogs from Groups IV and V that further indicated an aggregation of alpha tracks.

Electron Microscopy of Popliteal Lymph Nodes

The electron microscopic identification of plutonium particles in popliteal lymph nodes was accomplished in 3 ways. First, electron dense structures having the described appearance of plutonium (Adee and Sanders, 1968) were identified in specimens of popliteal lymph nodes that had alpha activity demonstrated autoradiographically with light microscopy. Since these structures were not seen in control popliteal lymph nodes from the uninjected sides, it is reasonable to identify these structures as plutonium. The two more direct ways of identification were with electron diffraction (Table 13, Figs. 21 and 22) and by electron microscopic autoradiography (Figs. 23 and 24). Particles identified by each of the 3 different ways had a similar appearance and were associated with similar types of cellular structures.

Table 12. Alpha Tracks in 6 Random Microscopic Fields of Left Popliteal Lymph Node Exposed to Autoradiographic Emulsion for 1 Hour

Group	Dog	Tracks/Source ($\bar{X}$ ± S. E.)
I (4 weeks)	2398	2.7 ± 0.4
	2515	1.9 ± 0.9
	2546	5.9 ± 2.3
	mean	3.5 ± 1.3
II (8 weeks)	2396	1.0 ± 0.0
	2509	6.8 ± 1.2
	2405	2.5 ± 0.4
	mean	3.4 ± 1.0
III (16 weeks)	2426	5.0 ± 0.7
	2536	4.8 ± 0.9
	2386	3.9 ± 0.5
	mean	4.5 ± 0.6
IV (32 weeks)	2403	1.9 ± 0.4
	2535	5.5 ± 0.6
	2527	9.4 ± 2.3
	mean	5.5 ± 0.8
V (32 weeks)	2406	6.3 ± 0.8
	2526	10.8 ± 2.1
	2397	11.9 ± 3.2
	mean	9.7 ± 1.1

Table 13. Electron Diffraction Crystallographic Planes for Plutonium Oxide

ASTM Reported		Observed Values*		
d	hkl	d^1	d^2	d^3
3.097	111	3.17	3.02	3.08
2.695	200	2.64	2.68	2.59
1.908	220	1.92	1.92	
1.627	311	1.62	1.68	1.58
1.558	222		1.58	1.50
1.349	400	1.36	1.35	1.35
1.238	331	1.24	1.25	1.22
1.207	420			
1.1015	422	1.10	1.10	1.12
1.0379	333	1.04	1.04	1.04
.95359	440		.966	.91
.91185	531			

*observed d-spacings calculated from $L\lambda$ (3.17) which has measured from diffraction patterns of pure aluminum standard.

d^1 as received (standard)

d^2 radioactive particles in stained popliteal lymph tissue (Fig. 22)

d^3 radioactive particles in unstained popliteal lymph tissue (Fig. 21)

The plutonium particles were aggregated in round to oval clear spaces within phagolysosomes of macrophages (Figs. 25, 26, and 27) and degenerate cells that could not be clearly identified (Figs. 28, 29, 30, 31, and 32). The plutonium particles were polygonal, had smooth or slightly serrated surfaces, and, although they were generally electron opaque, occasionally less dense appearing peripheral areas were present (Fig. 27). They were distinct and never intimately surrounded with adhering membrane structures or other electron dense material. The plutonium particles frequently appeared partially disoldged from the clear space containing them, possibly due to dislodgement during cutting. The clear spaces containing plutonium particles were not clearly lined with a membrane and the edges may have been spread apart by the electron beam. The phagolysosomal structures containing plutonium also contained other granular phagocytosed structures (Fig. 30) and lipofuscin (Figs. 26 and 27). In Fig. 26 a macrophage has apparently phagocytosed a cell containing plutonium. Myelin whorls were common in the cytoplasm of cells showing severe damage due to plutonium.

The surrounding scar tissue contained fibroblasts, and collagen fibers with the usual periodicity. A portion of a macrophage containing electron dense material resembling hemosiderin is shown (Fig. 33). Phagocytosed hemosiderin was a common histologic feature. The capillaries were unremarkable (Fig. 34).

External Iliac (Tables 14 & 15) and Superficial Inguinal Lymph Nodes

Compared to the popliteal lymph nodes, the external iliac lymph nodes from control dogs tended to have fewer lymphocytes in the cortical nodules and smaller germinal centers. A moderate amount of adipose tissue was present in dog 2515. The external iliac lymph nodes did not show any appreciable effects of amputation. A moderate arteritis involved one muscular artery in the right external iliac lymph node of dog 2509; this was probably an incidental finding and unrelated to plutonium deposition.

There were no changes attributable to plutonium in the external iliac lymph nodes from dogs in Groups I or II. External iliac lymph nodes from 2 dogs (Numbers 2536 and 2386) in Group III, dog 2527 in Group IV, and dog 2397 in Group V had areas of cortex replaced with foci of reticular cells and macrophages mixed with scattered necrotic debris. These foci were associated with fibrosis, neutrophilic infiltration, and hemosiderin deposition. The general morphology of the lymph nodes was intact and the uninvolved areas were essentially normal.

Autoradiographs exposed for 24 hours had single tracks in several left and right external iliac lymph nodes from Groups I and II. Alpha activity, generally composed of randomly distributed small stars in medullary cords, was present in several dogs from Groups II

Table 14. Histopathologic examination of left external iliac lymph node.

Dosage Group & Animal Number	Inflammatory Changes								Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis	autoradiographic activity	number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																						
2398	0	0	0	0	0	0	0	0^+	1-2	1	0	2	1	2	±	1	1	1	1	3	0	0
2515	0	0	0	0	0	0	0	0	2	2	2	2	1	1	1	2	1	1	1	0	0	1
2546	0	0	0	0	0	0	0	0	1	0	0	2	1	1	±	0	2	1	1	0	0	0
Group II (8 weeks)																						
2396	0	0	0	0	0	0	0	0^+	1-2	1	0	2	2	1	0	±	1	1	±	±	±	±
2509	0	0	0	0	0	0	0	1	2	2	±	1-2	1	1	1	±	1	1	±	1	0	0
2405	0	0	0	0	0	0	0	0^+	1-2	1	0	2	2	±	1	1	2	1	±	±	0	±
Group III (16 weeks)																						
2426	0	0	0	0	0	0	0	±	1	1	1	2	2	1	1	1	2	1	±	1	0	0
2536	0	1	0	±	1	0	0	2	2	2	0	1	1	1	1	1	2	1	1	1	±	1
2386	1	±	±	±	±	0	0	2	2	2	0	2	2	1	1	±	1	1	±	±	1	1
Group IV (32 weeks)																						
2403	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	±	2	1	1	±	±	0
2535	0	0	0	0	0	0	0	±	2	1	0	2	2	1	±	1	1	1	±	1	0	±
2527	0	0	0	0	0	0	0	1	2	1	±	2	1	±	1	±	2	1	1	1	0	0
Group V (32 weeks)																						
2406	0	0	0	0	0	0	0	1	1	±	0	2	1	1	1	±	2	1	±	±	±	±
2526	0	0	0	0	0	0	0	±	2	1-2	0	2	2	±	±	±	2	1	±	1	±	±
2397	±	1	1	1	±	0		±-1	1-2	2	0	2	2	1	1	1	2	1	±	±	±	1
Controls (8 weeks)																						
2540	0	0	0	0	0	0	0	0	2	±	0	1	1	1	±	1	2	1	±	±	±	±
(16 weeks)																						
2519	0	0	0	0	0	0	0	0	2	1-2	1	2	2	2	1	1	2	2	±	0	±	0
(32 weeks)																						
2541	0	0	0	0	0	0	0	0	2	±	1	2	2	1	1	±	2	1	±	0	1	2
(32 weeks)																						
2534	0	0	0	0	0	0	0	0	2	2	±	1-2	2	1	±	1	1-2	1	±	0	0	0

Key =
- 0 = none
- 0^+ = trace
- ± = very slight (or very small amount)
- ±-1 = very slight-to-slight (or very small-to-small amount)
- 1 = slight (or small amount)
- 1-2 = slight-to-moderate (or small-to-moderate amount)
- 2 = moderate
- 2-3 = moderate-to-marked
- 3-4 = marked-to-extreme
- 4 = extreme

Table 15. Histopathologic examination of right external iliac lymph node.

Dosage Group & Animal Number	Inflammatory Changes								Cortex			Medullary Cords					Medullary Sinusoids					
	scar formation	increased numbers of reticular cells and macrophages	cellular infiltration	necrosis	pigment	hemorrhage	necrotic vasculitis	autoradiographic activity	number of follicles	germinal center size	adipose tissue	thickness	plasma cells	lymphocytes	neutrophils	pigment	macrophages	lymphocytes	neutrophils	hemorrhage	erythrophagocytosis	pigment
Group I (4 weeks)																						
2398	0	0	0	0	0	0	0	0^+	2	1	0	2	1	2	±	1	2	1	1	0	0	0
2515	0	0	0	0	0	0	0	0^+	2	1	1	2	1	1	1	1	2	1	1	±	0	1
2546	0	0	0	0	0	0	0	0	2	2	0	1	1	1	±	±	1	1	±	1	0	±
Group II (8 weeks)																						
2396	0	0	0	0	0	0	0	0	2	1	±	1	1	1	±	±	1	1	±	1	0	0
2509	0	0	0	0	0	0	2	0	2	1	0	1	1	1	1	0	1-2	1	±	0	0	0
2405	0	0	0	0	0	0	0	±	1	±	0	1-2	1	1	1	±	2-3	1	±	1	0	±
Group III (16 weeks)																						
2426	0	0	0	0	0	0	0	0	1	±	0	1	1	1	1	±	2	2	±	2	0	0
2536																						
2386																						
Group IV (32 weeks)																						
2403	0	0	0	0	0	0	0	0	1	±	0	1	1	1	1	0	2	1	1	±	±	0
2535	0	0	0	0	0	0	0	0	2	1-2	0	2	2	1	±	±	1	1	±	1	0	0
2527	0	1	±	1	0	0	0	1	1	±	0	1	0	1	0	0	3	1	0	±	0	0
Group V (32 weeks)																						
2406	0	0	0	0	0	0	0	0	1	±	1	2	1	1	1	±	2	1	±	±	0	0
2526	0	0	0	0	0	0	0	0	2	2	0	1-2	2	1	±	±	2	1	±	±	±	0
2397	0	0	0	0	0	0	0	±	2	2	0	2	2	1	1	±	2	1	±	±	±	±
Controls (8 weeks)																						
2540	0	0	0	0	0	0	0	0	2	2	0	1	1	1	±	0	2-3	1	±	0	0	0
(16 weeks)																						
2519	0	0	0	0	0	0	0	0	2	±	0	1-2	1	1	±	0	2	2	±	0	0	0
(32 weeks)																						
2541	0	0	0	0	0	0	0	0	2	1	1	2	2	1	1	±	2	1	±	0	±	±
(32 weeks)																						
2534	0	0	0	0	0	0	0	0	1-2	2	0	2	2	1	±	0	2-3	1	±	0	0	0

Key =
- 0 = none
- 0^+ = trace
- ± = very slight (or very small amount)
- ±-1 = very slight-to-slight (or very small-to-small amount)
- 1 = slight (or small amount)
- 1-2 = slight-to-moderate (or small-to-moderate amount)
- 2 = moderate
- 2-3 = moderate-to-marked
- 3-4 = marked-to-extreme
- 4 = extreme

through V. The plutonium particles present in the external iliac lymph nodes were clearly smaller and less numerous than present in the popliteal lymph nodes.

The right superficial inguinal lymph node from dog 2397 in Group V (32 weeks) had moderate sized areas of cortex replaced by increased numbers of reticular cells and macrophages that were associated with fibrosis, neutrophilic infiltration, scattered necrotic debris, and hemosiderin deposition. The 24 hour autoradiograph had a small to moderate amount of alpha activity composed of stars in the area of cortex replacement.

Fig. 1. Radioactivity in right hind paws injected with plutonium as measured by an external wound counter.

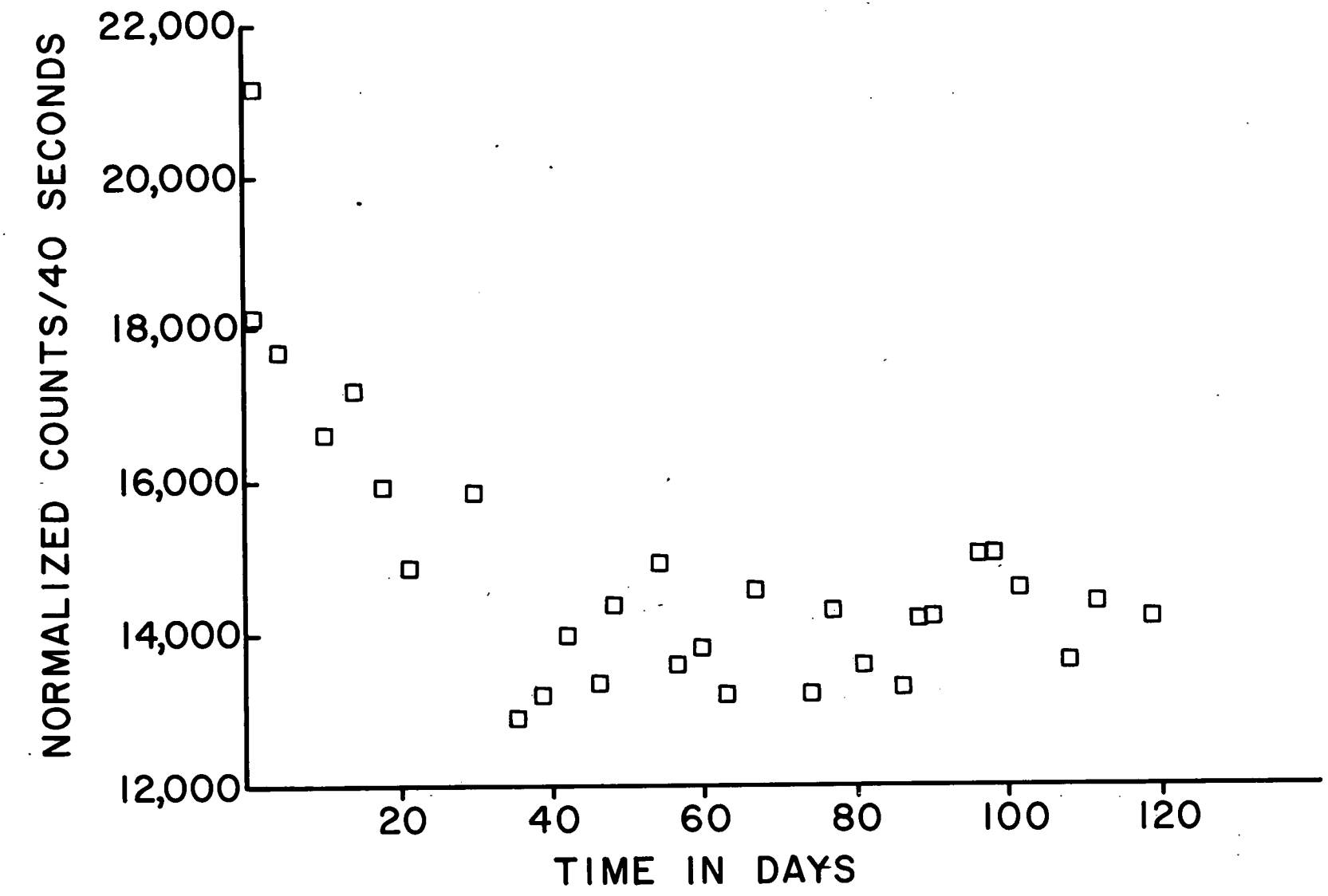

NORMALIZED COUNTS/40 SECONDS
22,000
20,000
18,000
16,000
14,000
12,000
20
40
60
80
100
120
TIME IN DAYS

Fig. 2. Radioactivity in the left popliteal lymph nodes of dogs from Group II as measured by an external wound counter.

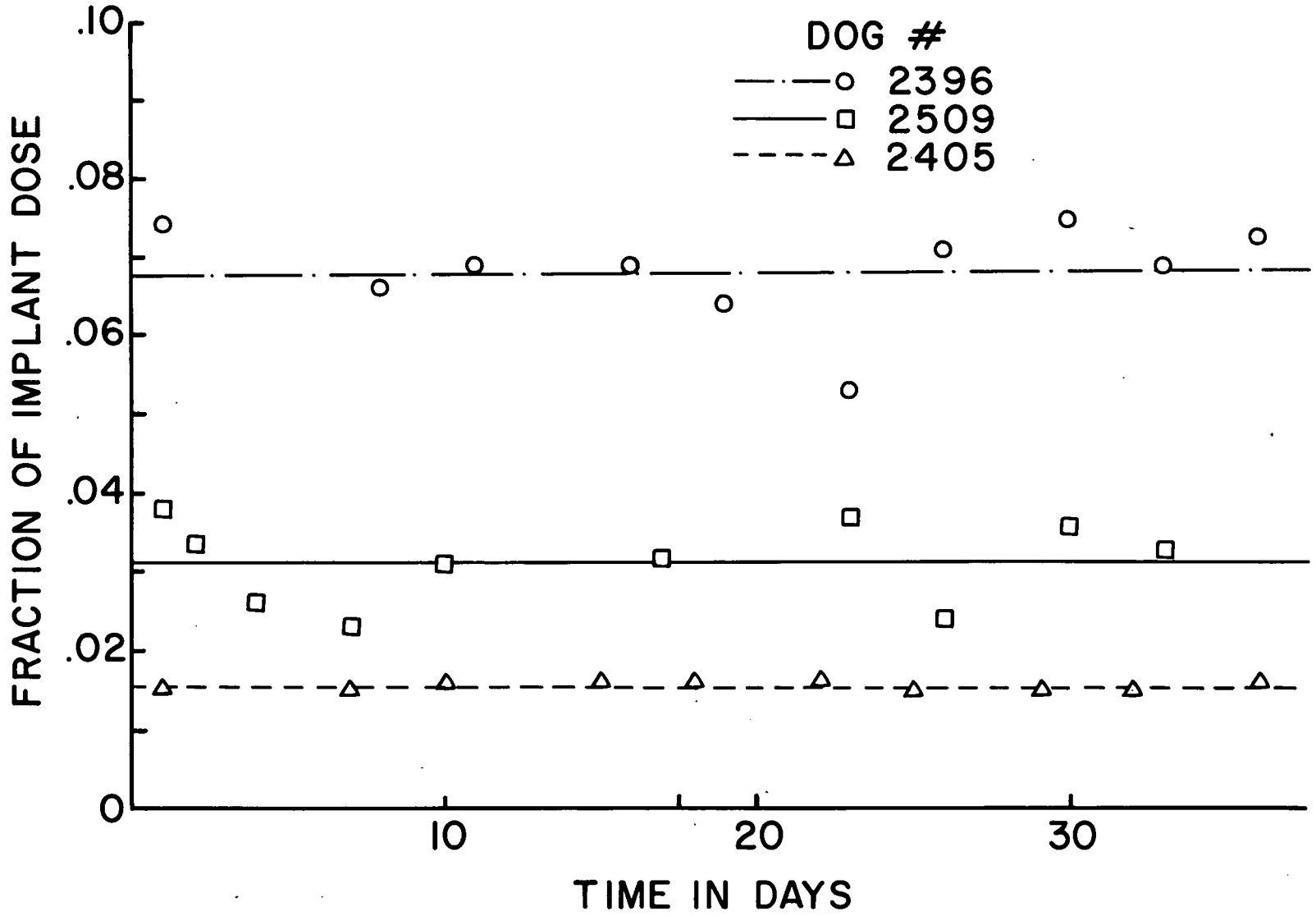

DOG #
2396
2509
2405
.10
.08
.06
.04
.02
0
FRACTION OF IMPLANT DOSE
10
20
30
TIME IN DAYS

Fig. 3. Radioactivity in the left popliteal lymph nodes of dogs from Group III as measured by an external wound counter.

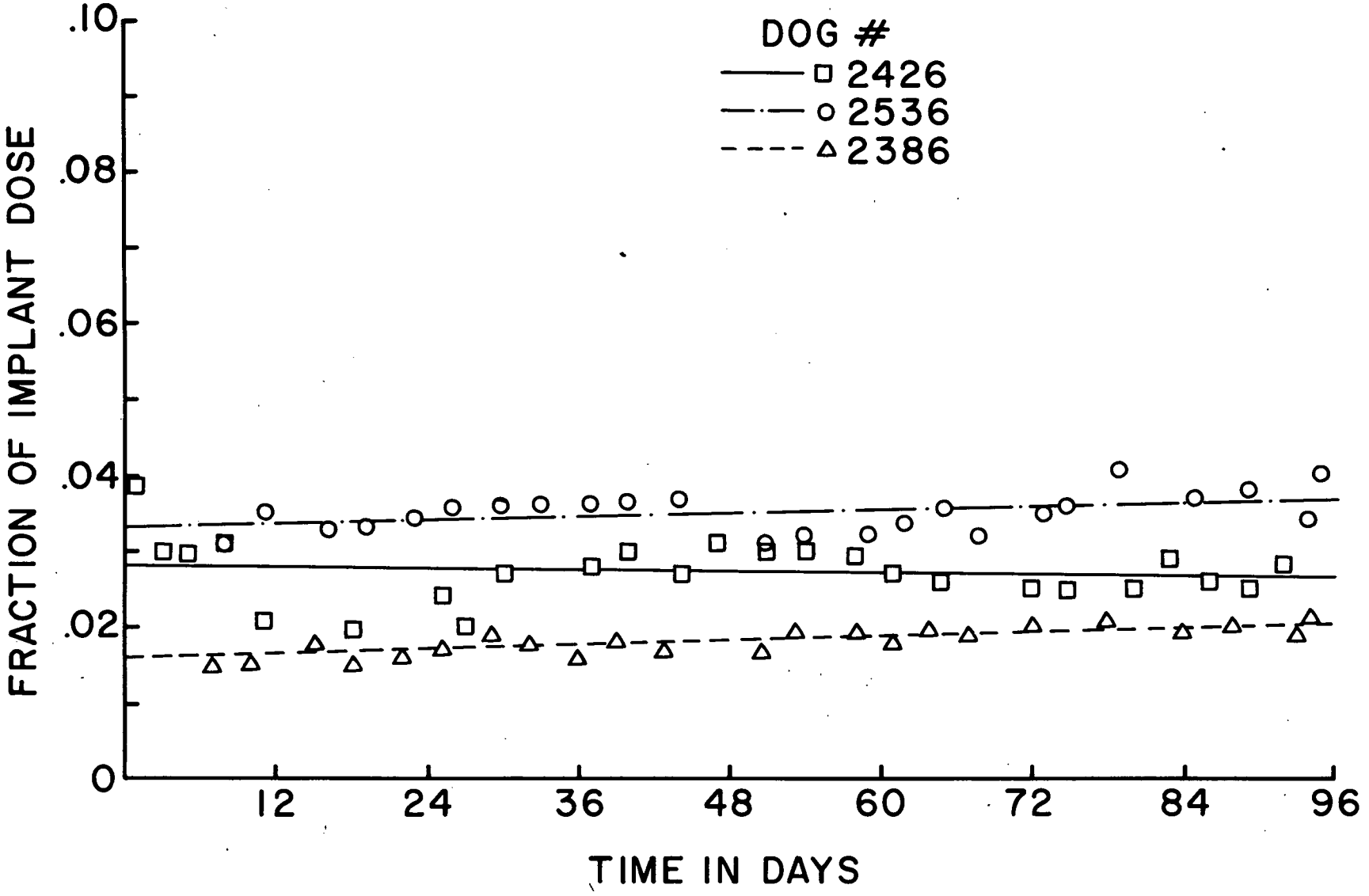

DOG #
2426
2536
2386
FRACTION OF IMPLANT DOSE
.10
.08
.06
.04
.02
0
12
24
36
48
60
72
84
96
TIME IN DAYS

Fig. 4. Radioactivity in the left popliteal lymph nodes of dogs from Group IV as measured by an external wound counter.

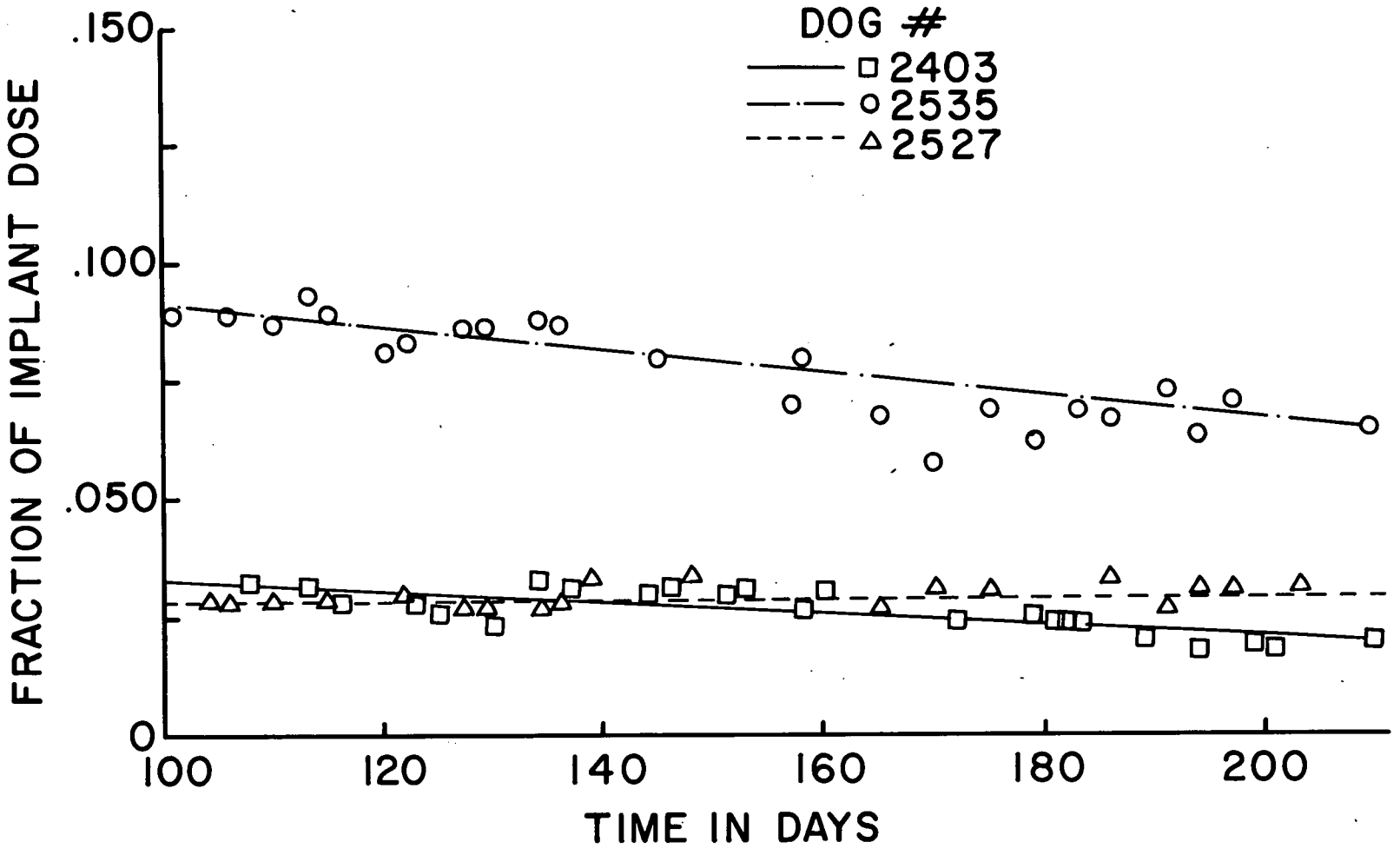

DOG #
2403
2535
2527
.150
.100
.050
0
FRACTION OF IMPLANT DOSE
100
120
140
160
180
200
TIME IN DAYS

Fig. 5. Radioactivity in the left popliteal lymph nodes of dogs from Group V as measured by an external wound counter.

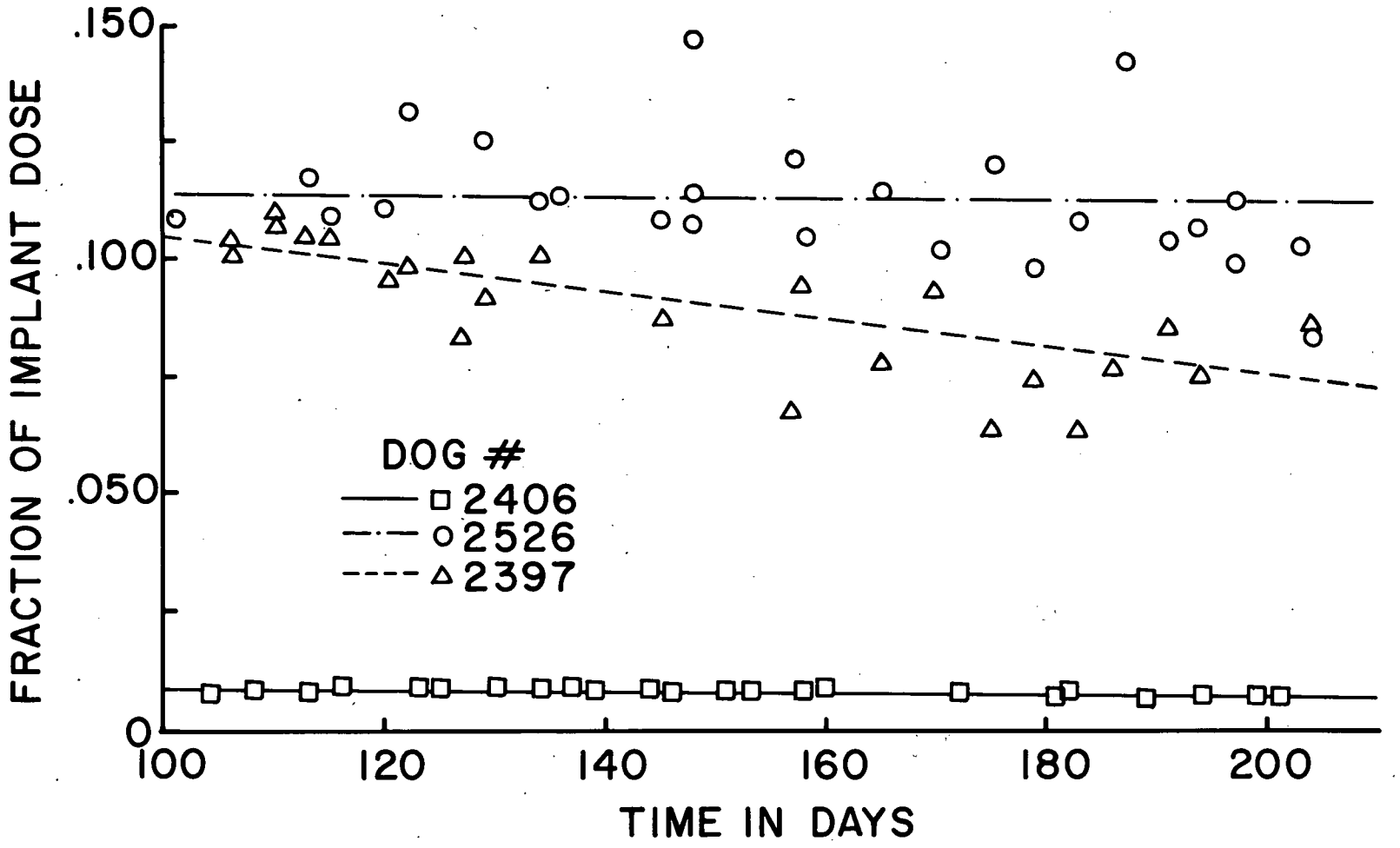

.150
.100
.050
0
FRACTION OF IMPLANT DOSE
100
120
140
160
180
200
TIME IN DAYS
DOG #
2406
2526
2397

Fig. 6. Radioactivity in the right popliteal lymph nodes of dogs from Groups II, III, IV, and V as measured by an external wound counter.

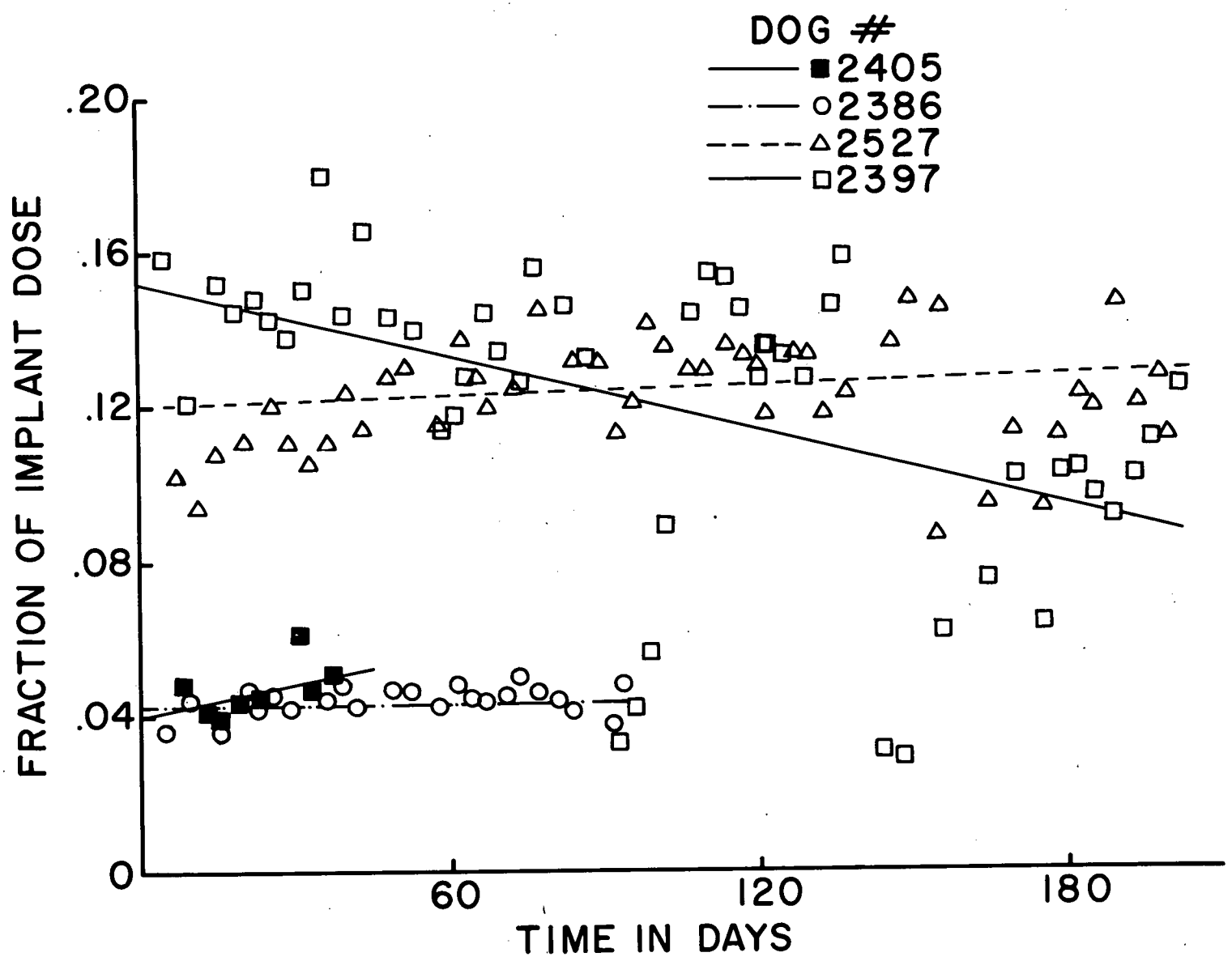
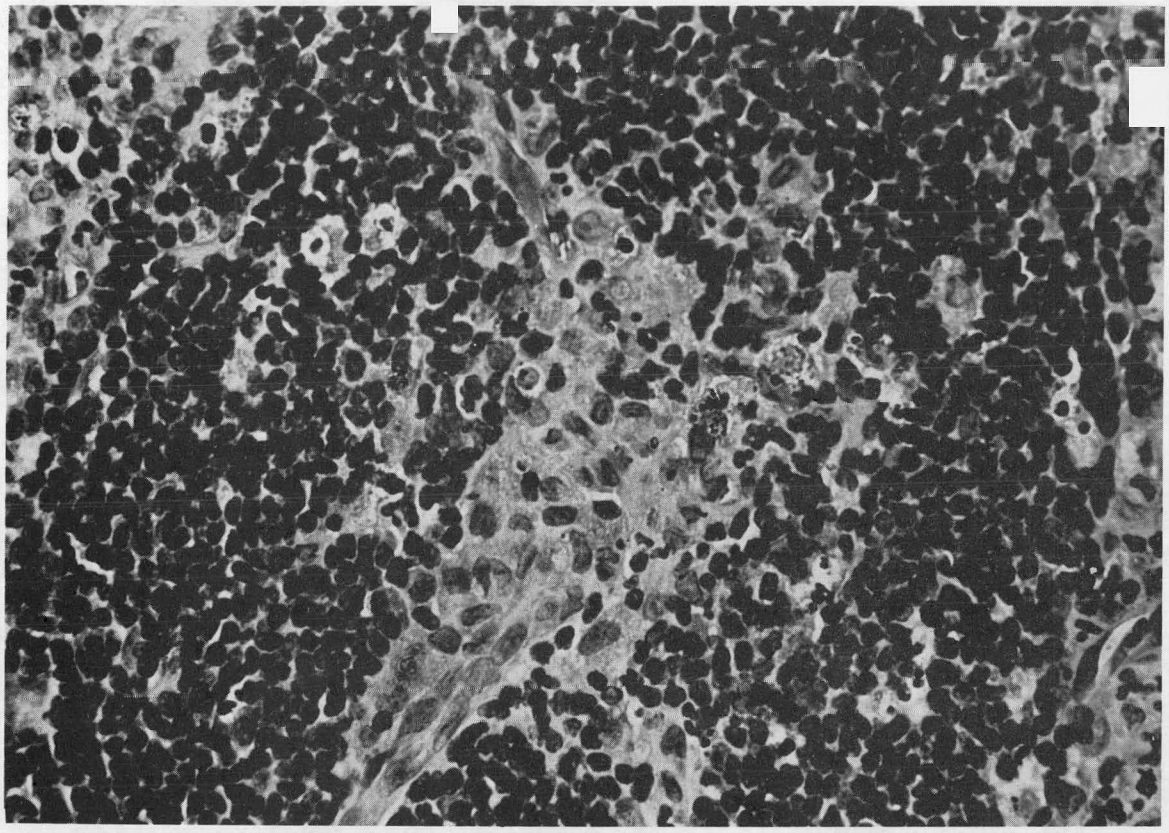

DOG #
2405
2386
2527
2397
.20
.16
.12
.08
.04
0
FRACTION OF IMPLANT DOSE
60
120
180
TIME IN DAYS

Fig. 7. This popliteal lymph node has a cortical lymphoid nodule with a well-demarcated germinal center containing necrotic debris and large reticular cells. X500.

Fig. 8. This field, adjacent to the lymphoid nodule in Fig. 7, is composed primarily of reticular cells mixed with a few neutrophils and macrophages. The macrophages frequently contain phagocytosed pyknotic debris. Some of the necrotic debris may be extracellular. X500.



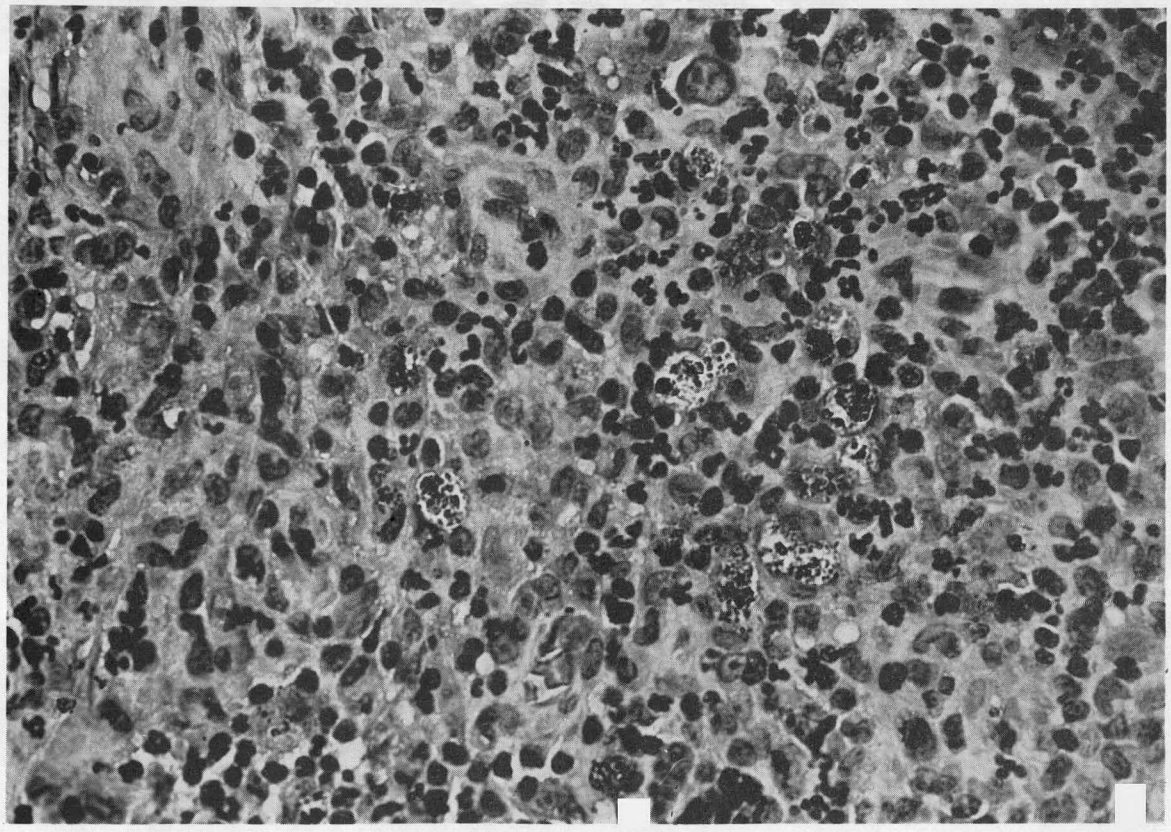


Fig. 9. Large areas of the cortex of this popliteal lymph node containing plutonium are replaced by reticular cell hyperplasia, increased numbers of macrophages, and early fibroplasia. X24.

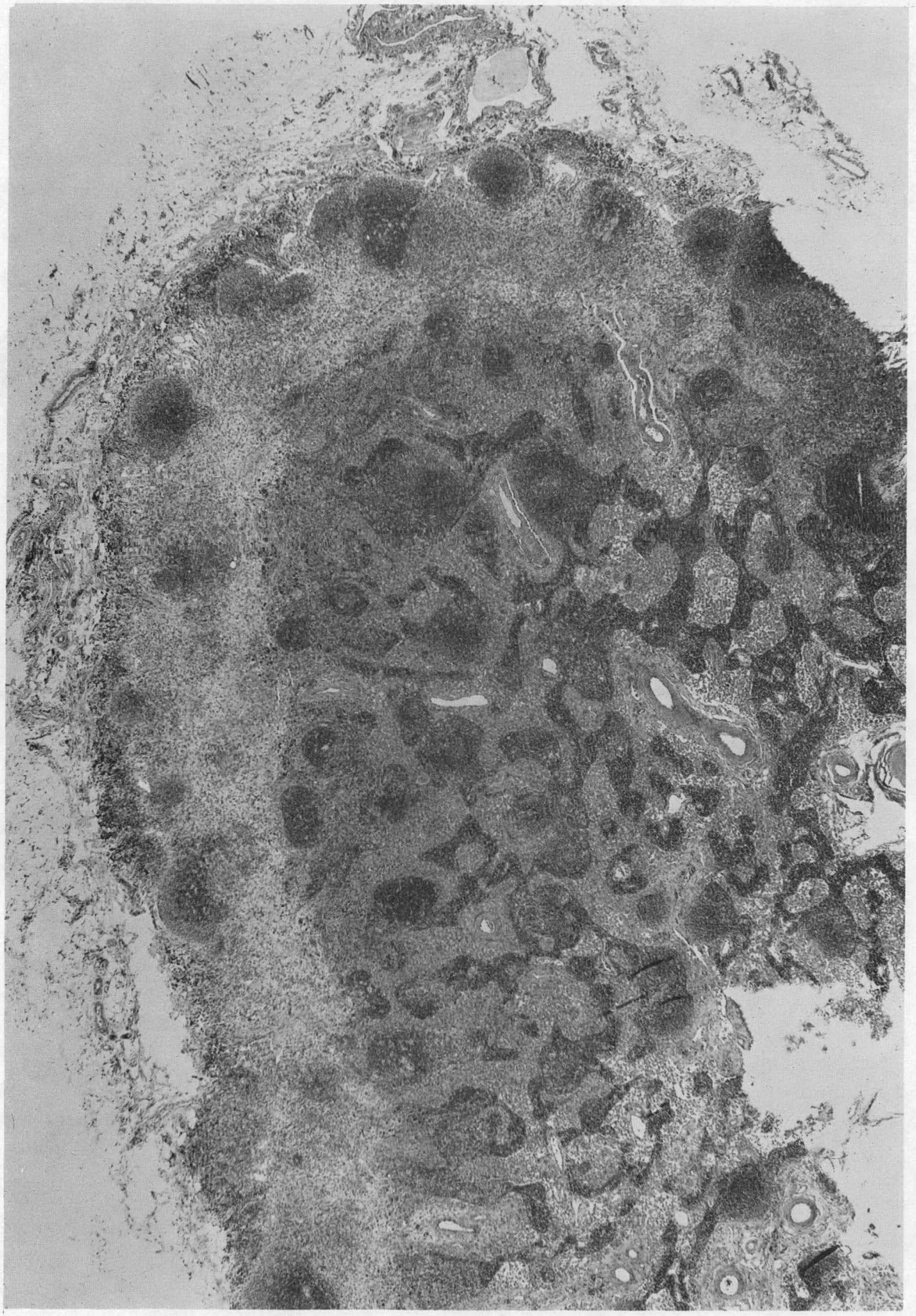


Fig. 10. The normal architecture of this popliteal lymph node containing plutonium is completely replaced by scar tissue. X16.

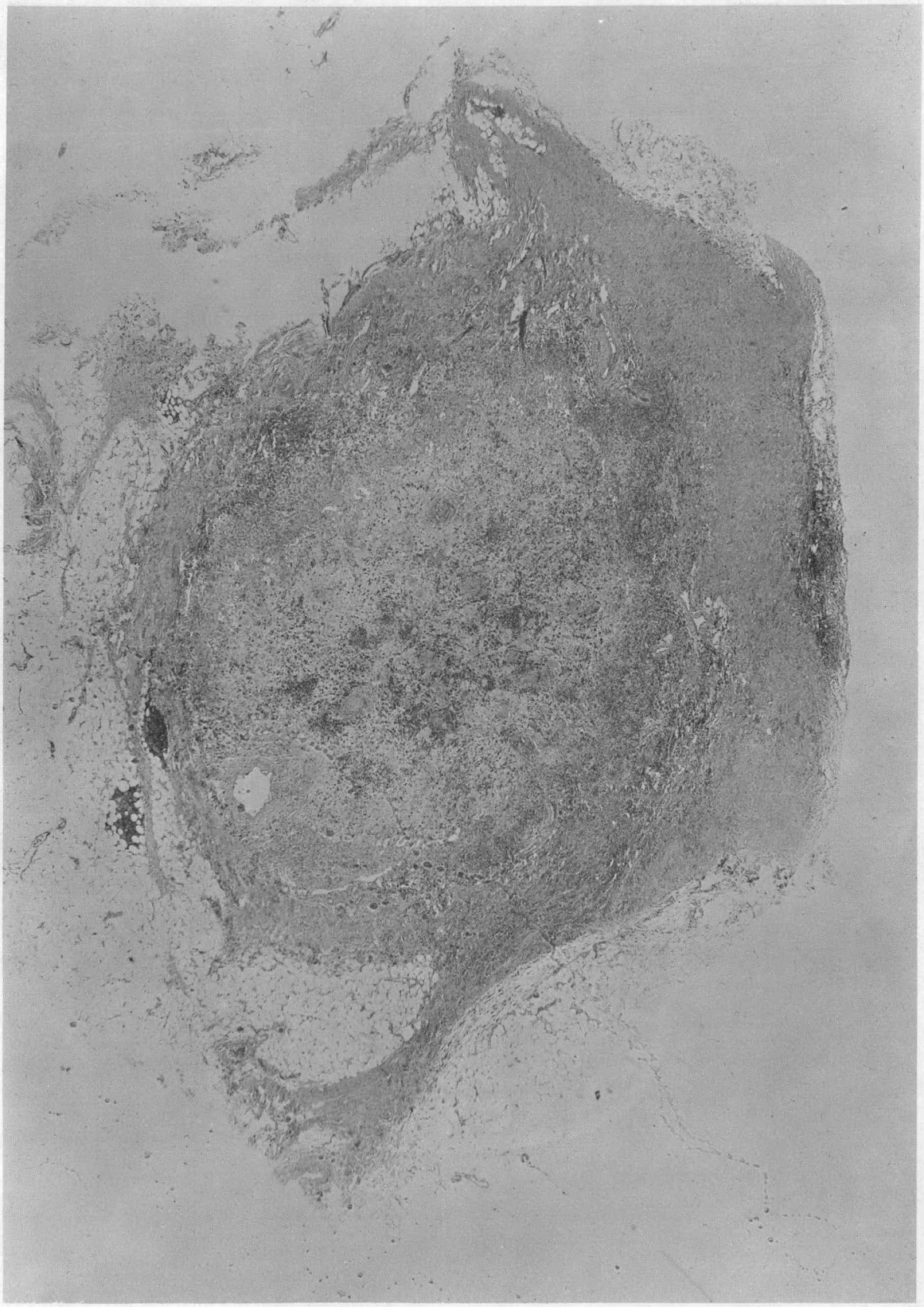
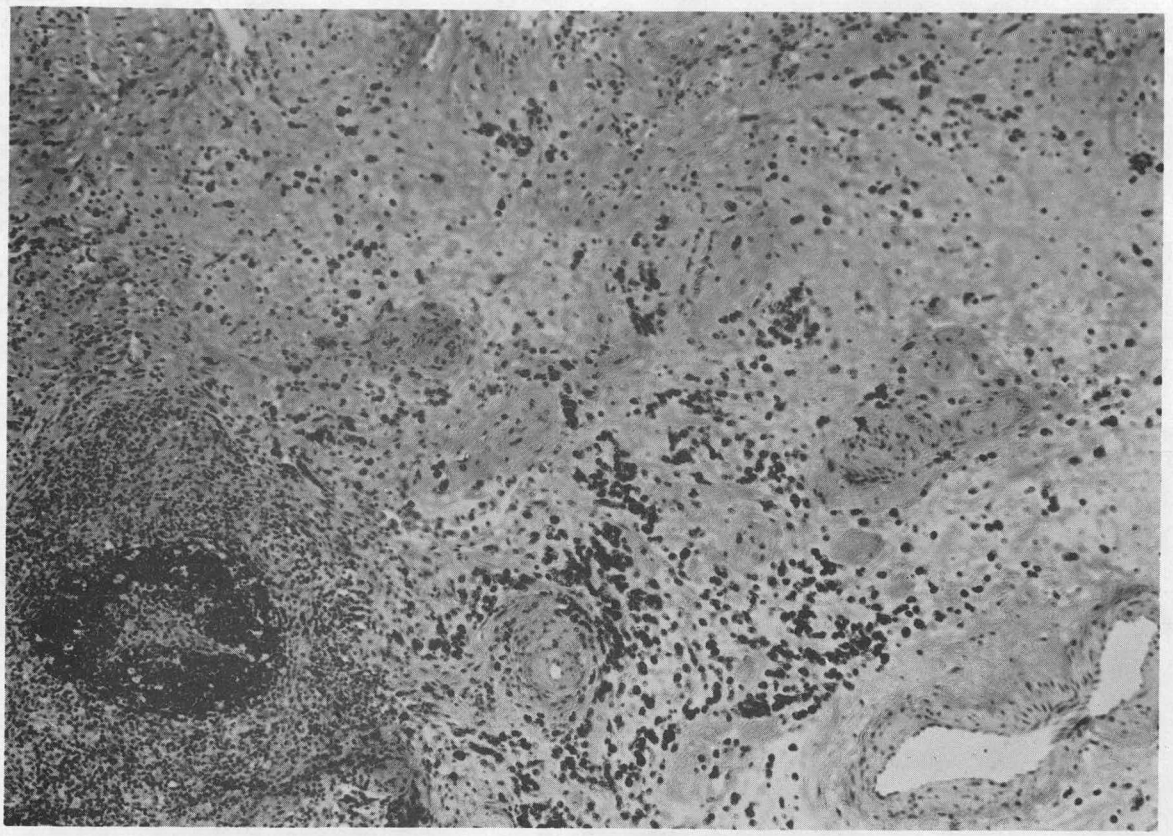


Fig. 11. The cortical lymphoid nodule from Fig. 7 is at the left but the normal morphology of the medulla, towards the right side of the figure, is largely replaced by necrotic debris and scar tissue. The tangential sectioned artery at the lower right is unremarkable. A moderate amount of hemosiderin, represented by darkly stained granules, is scattered throughout the nodule. X80.

Fig. 12. This is an autoradiograph near the same area shown in Fig. 11. Numerous large alpha stars are disseminated throughout the medulla. The alpha stars are numerous in areas of hemosiderin deposition, but are not restricted to these areas. This section was exposed to Ilford K-5 emulsion for 24 hours and developed with Dektol. X80.



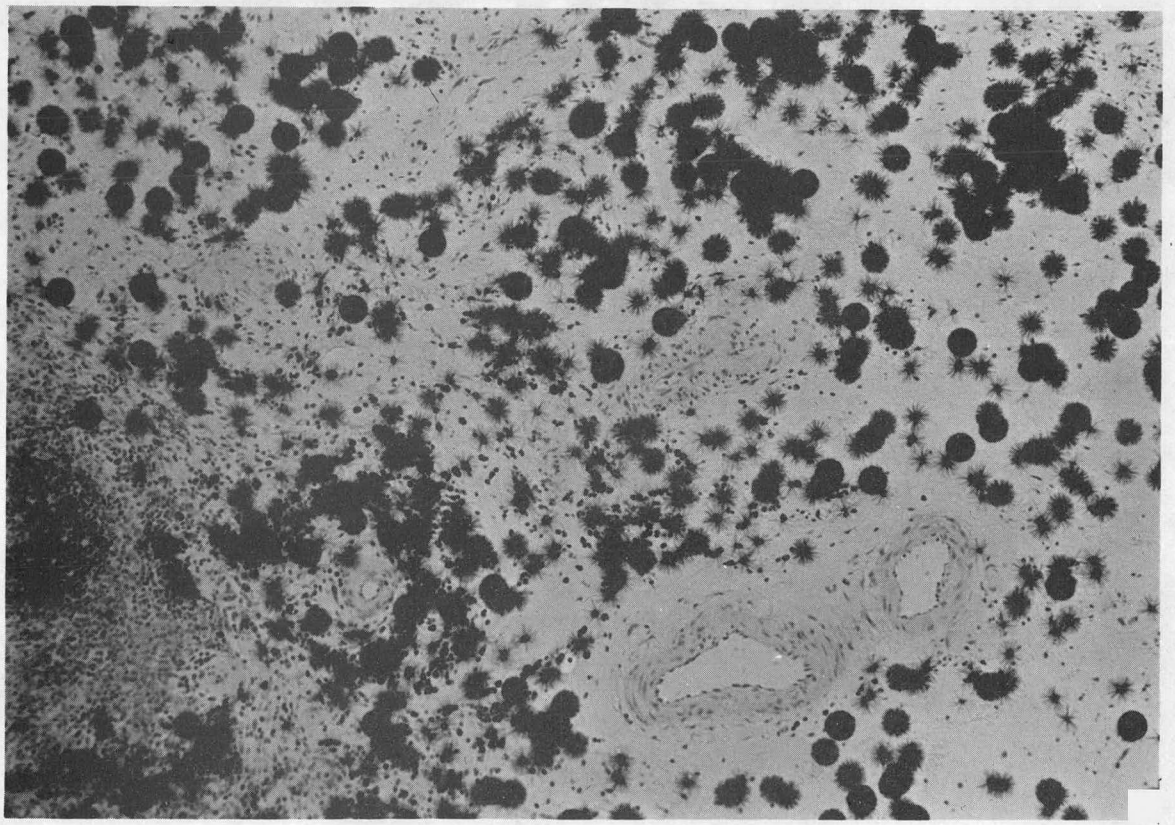


Fig. 13. This popliteal lymph node is replaced by loose scar tissue encapsulated by dense connective tissue. The loose scar tissue contains cavities with only a minimal amount of fibrin and necrotic debris. X24.

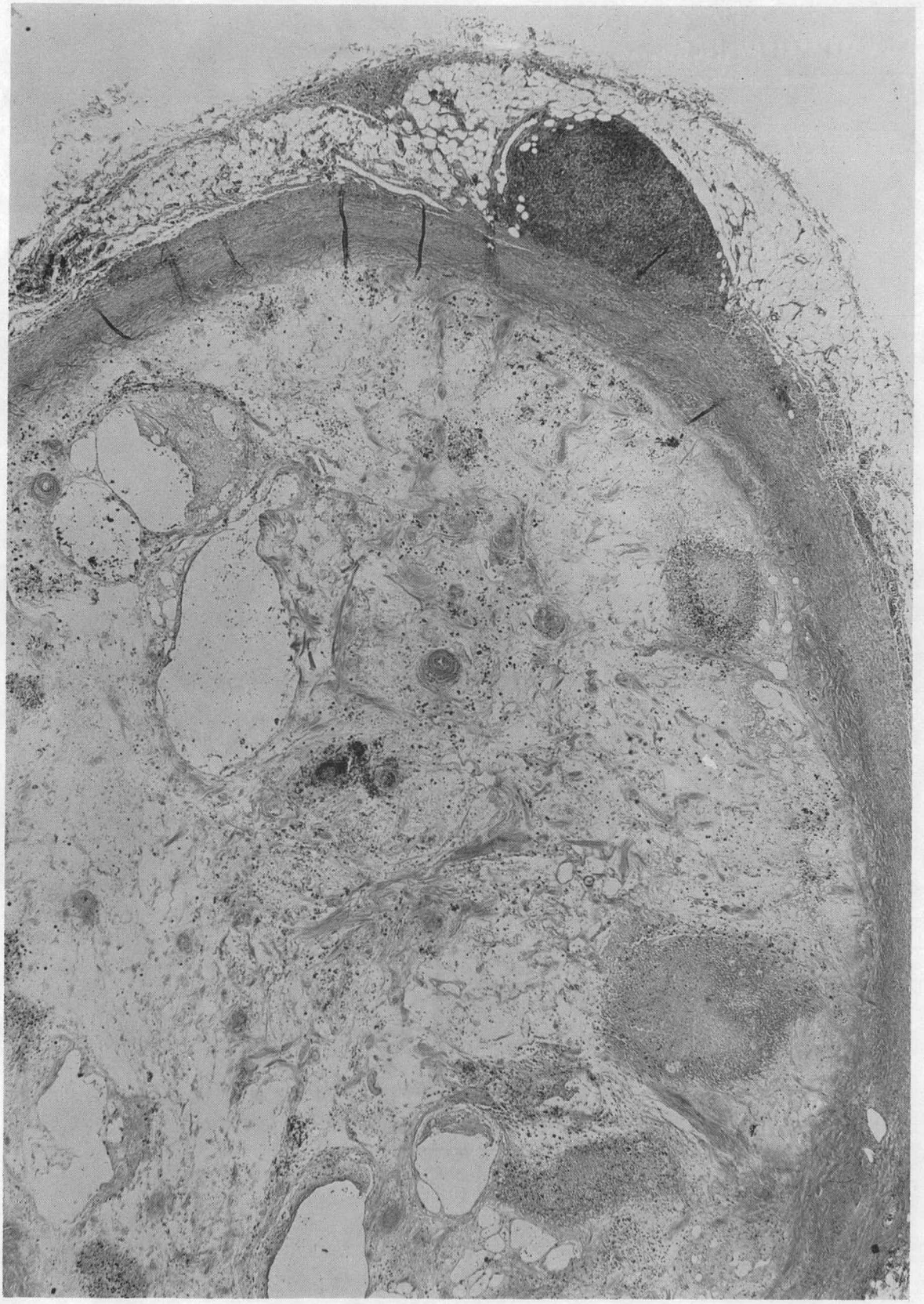
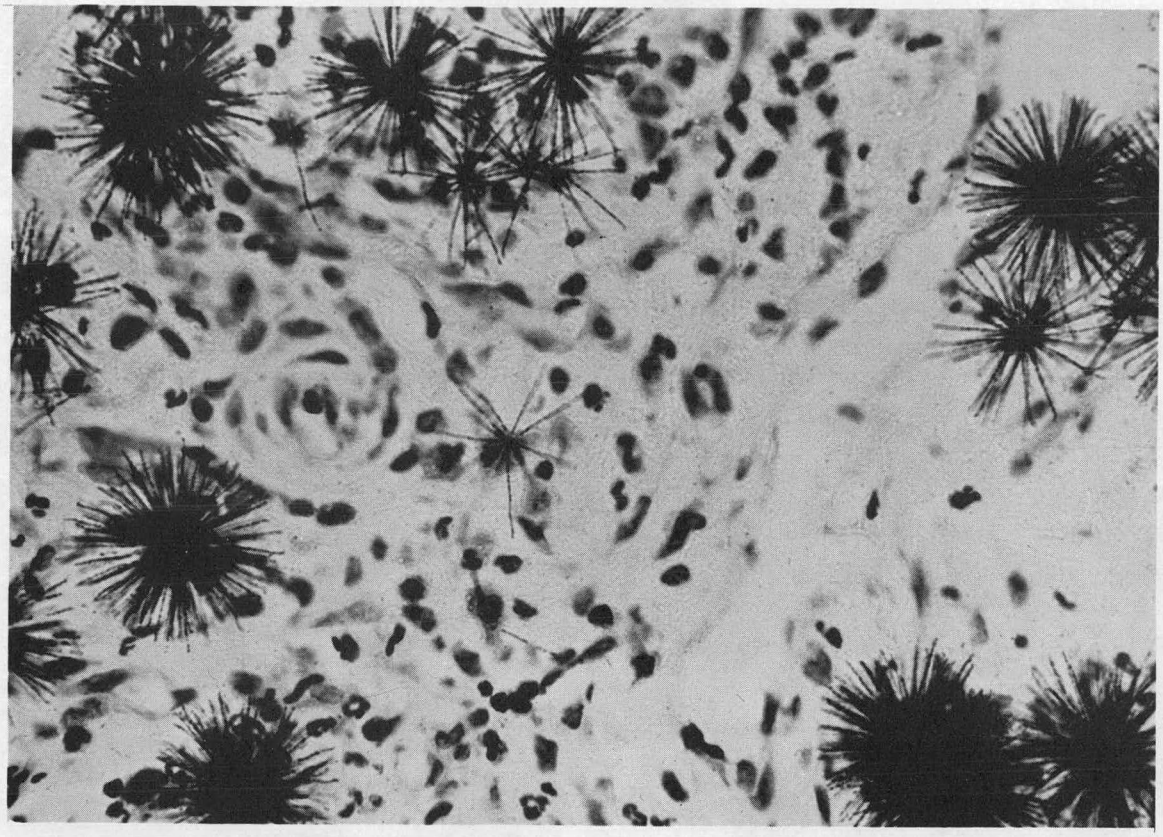


Fig. 14. Autoradiograph from an area of scar tissue composed primarily of fibroblasts, reticular cells, macrophages, a few neutrophils, and an abundant amount of intercellular matrix. The alpha stars are variable in size. The photograph is focused on the alpha tracks. Stained section exposed to Ilford K-5 emulsion for 24 hours and developed with Dektol. X500.

Fig. 15. Same photograph as Fig. 14 except the cells, instead of the alpha tracks, are in focus. X500.



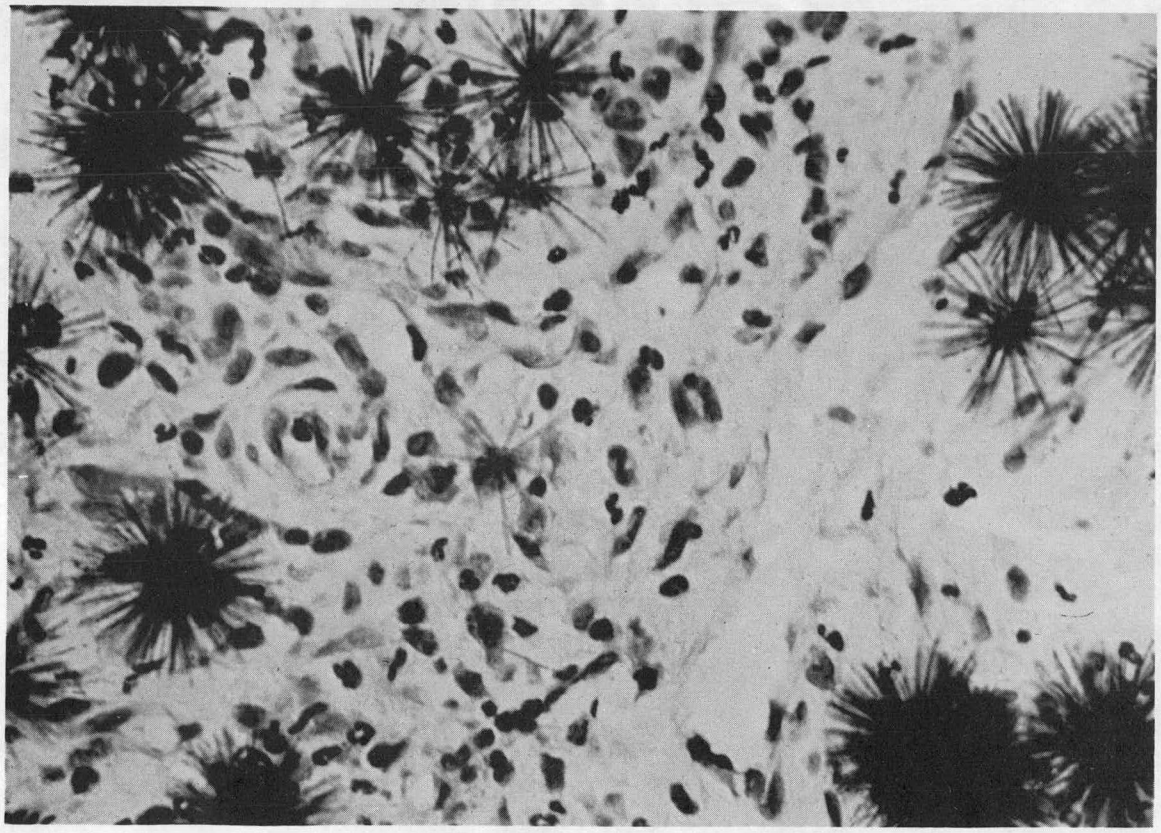
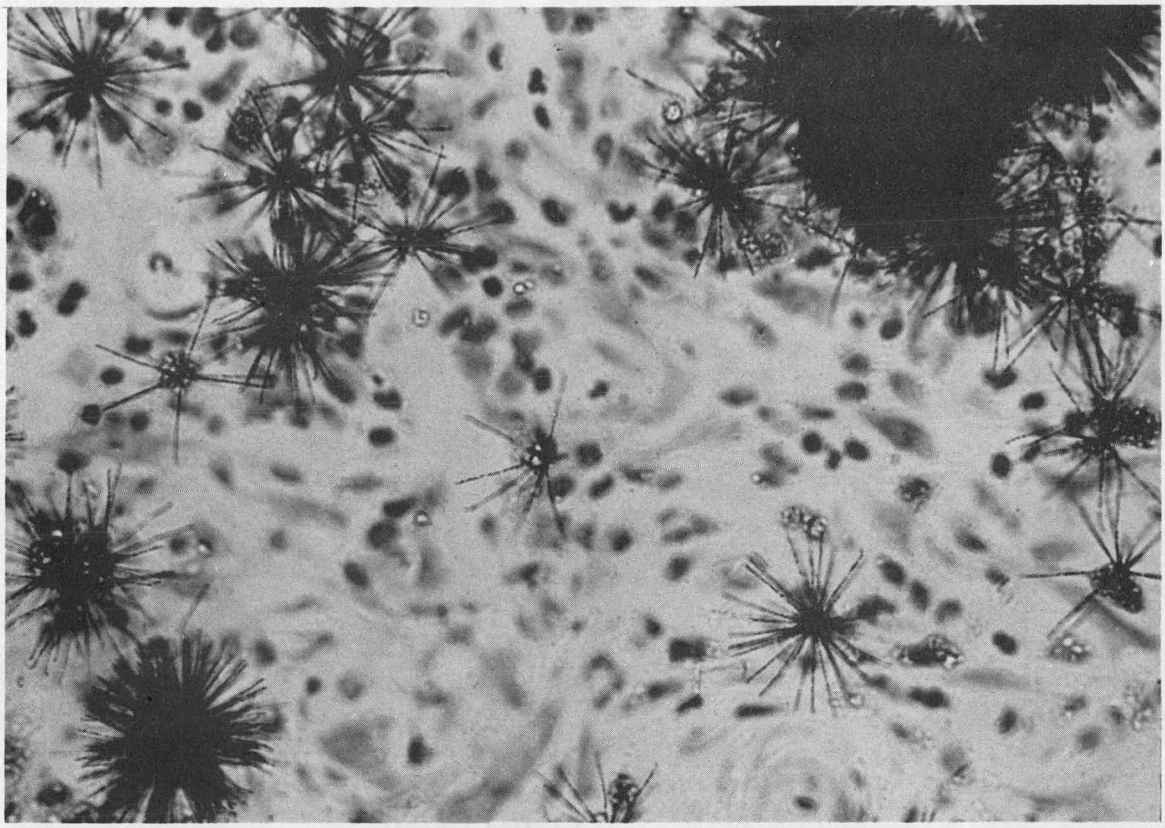


Fig. 16. This autoradiograph shows alpha stars of various sizes. The smaller alpha stars can be seen originating from cells containing granular particles; with special stains this material can be identified as hemosiderin. The larger alpha stars obliterate their source of origin. Some pigment granules are not associated with alpha tracks, but a longer exposure may have demonstrated tracks. Section exposed to Ilford K-5 emulsion for 24 hours and developed with Dektol. X500.

Fig. 17. Same photograph as Fig. 16 except the cells, instead of the alpha tracks, are in focus. X500.



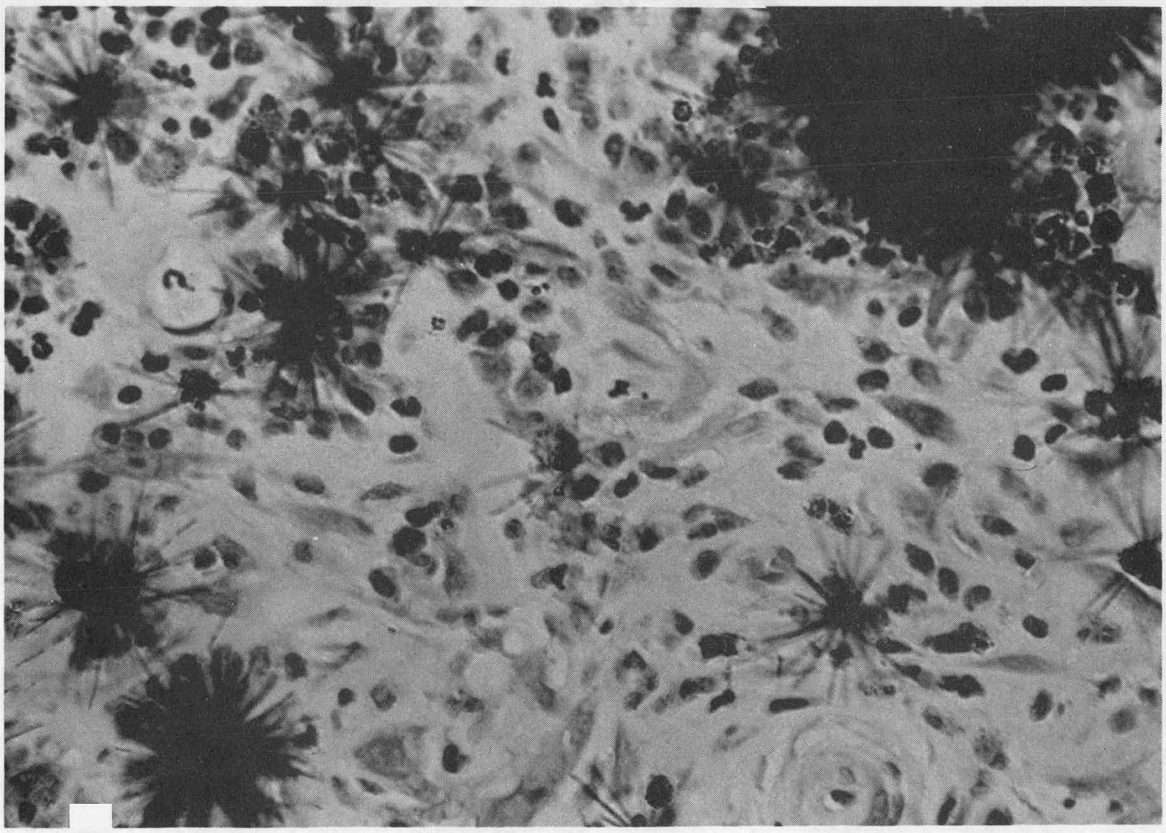
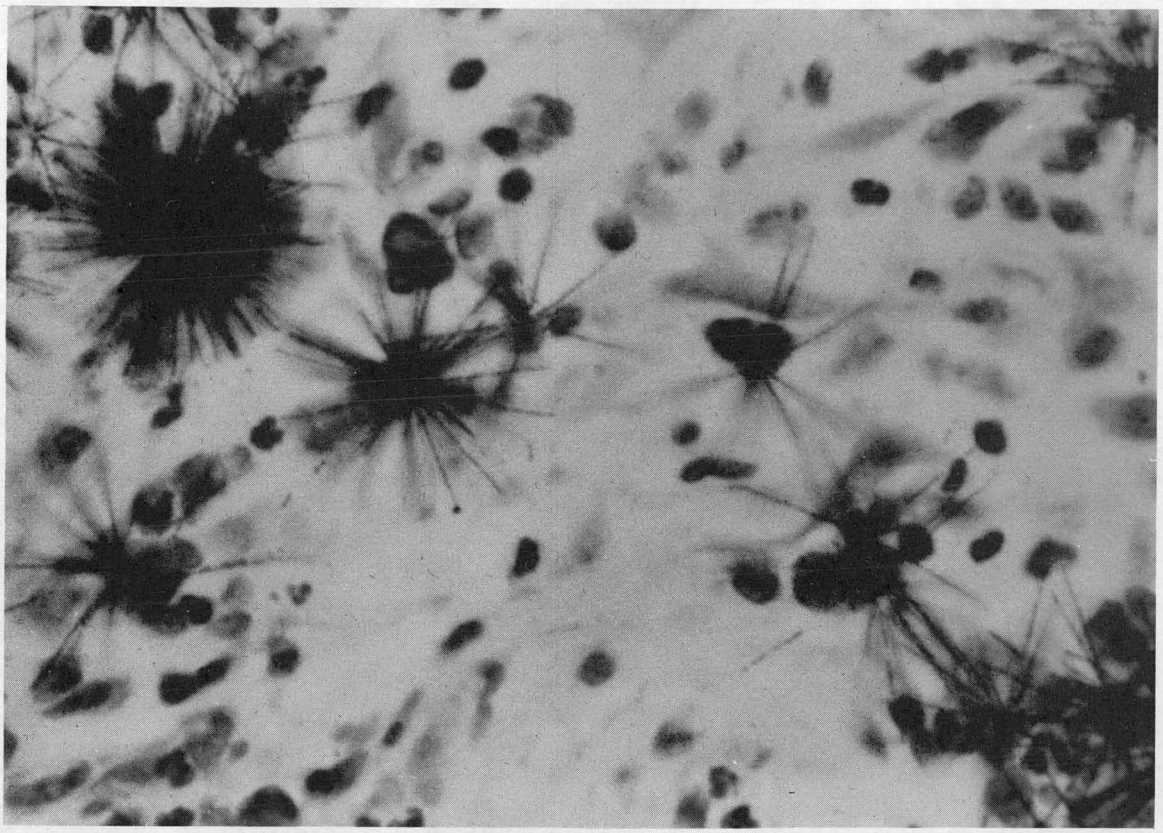


Fig. 18. This autoradiograph shows numerous alpha stars of variable size. One small star located to the right of center originates from a macrophage lacking discernible pigment. Section exposed to Ilford K-5 emulsion for 24 hours and developed with Dektol. X800.

Fig. 19. This autoradiograph shows small alpha stars originating from macrophages lacking any discernible pigment. Section exposed to Ilford K-5 emulsion for 24 hours and developed with Dektol. X800.



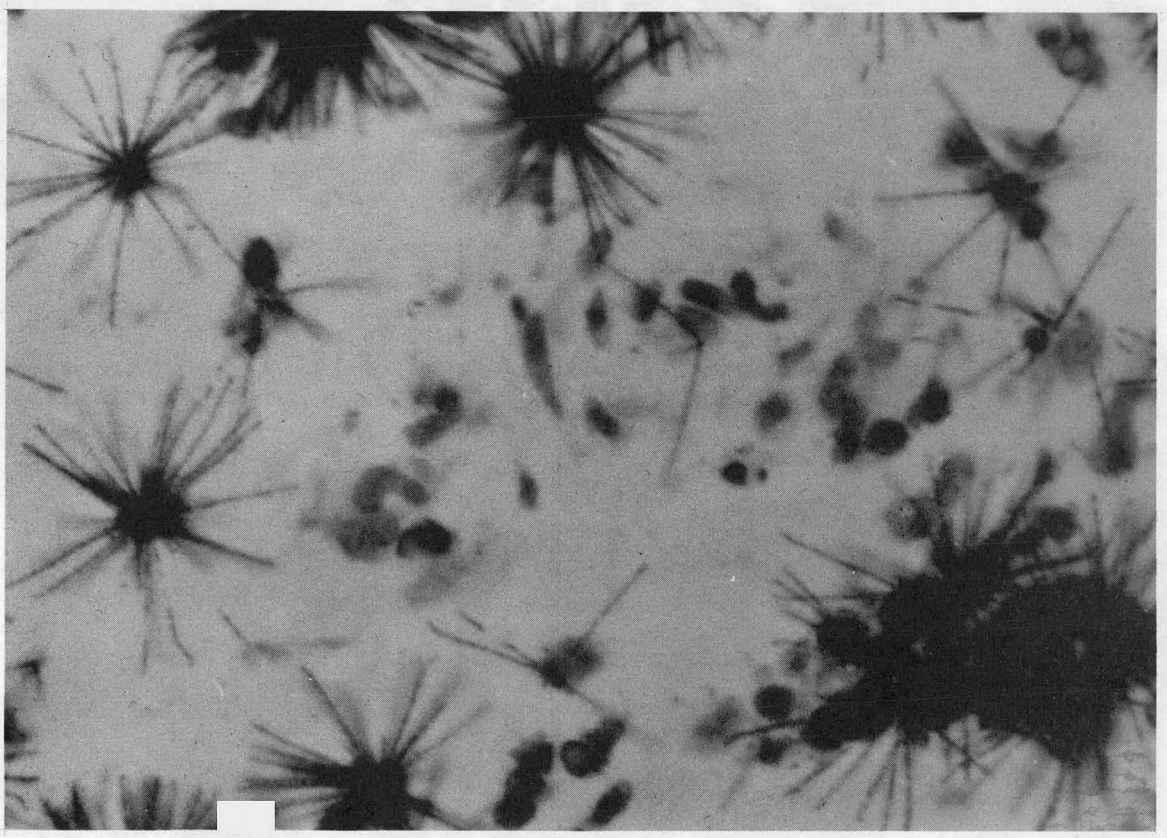


Fig. 20. The mean number of alpha tracks per source in random microscopic fields of left popliteal lymph node is plotted for each group of dogs. The linear regression analysis showed a curve of $y = 2.43 + 0.157\ t$ (t = weeks post exposure) with a slope significantly greater than zero ($P < 0.05$).

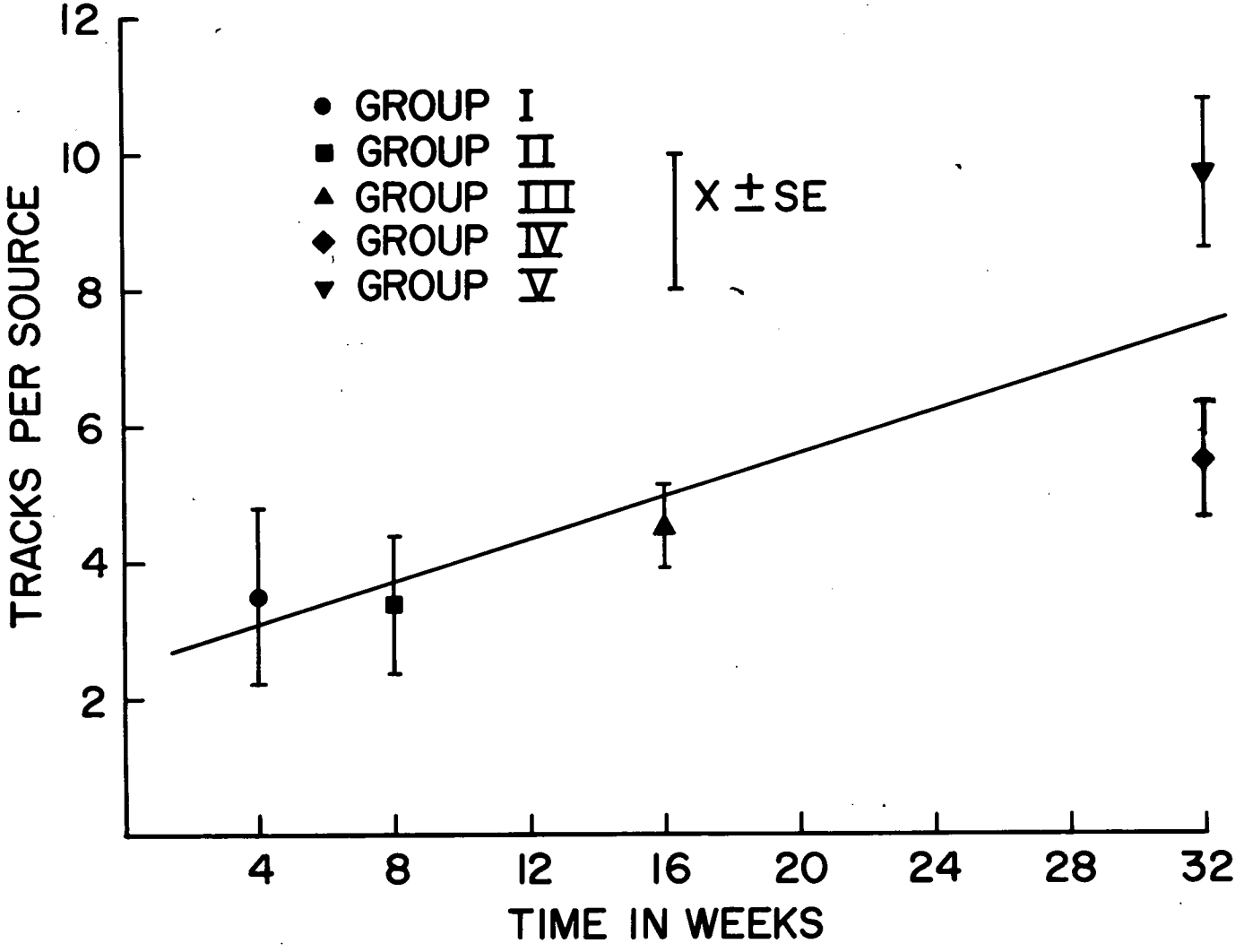
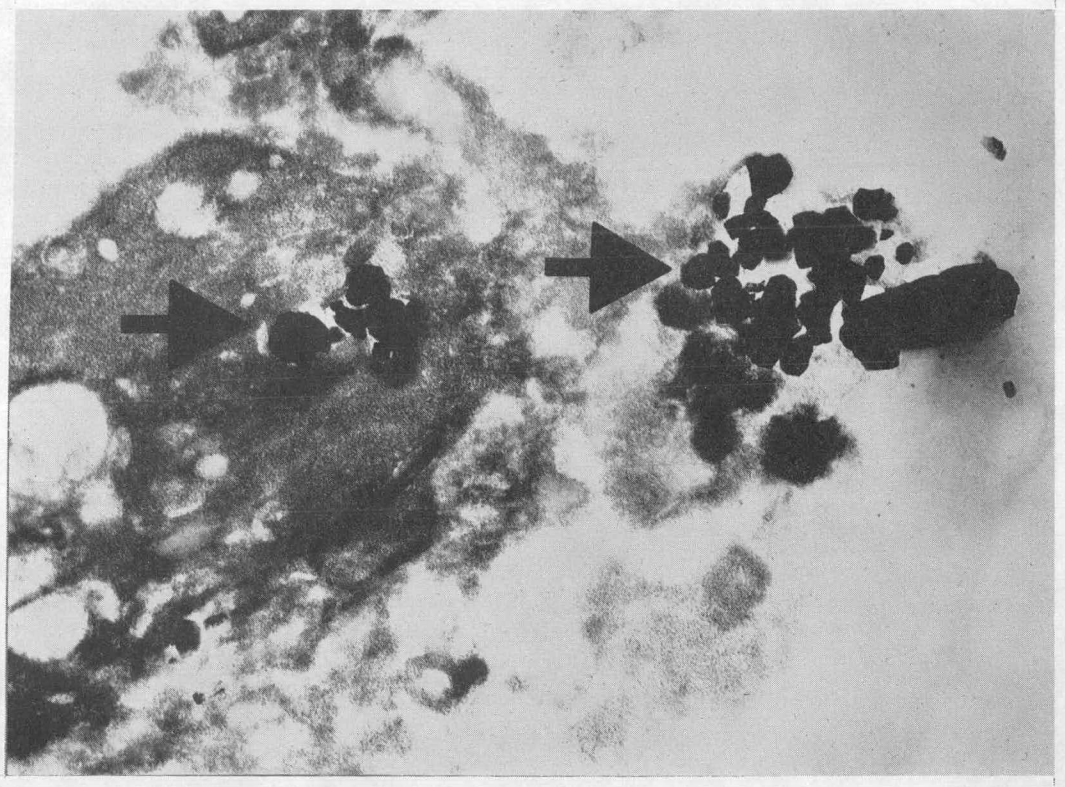

12
10
8
6
4
2
TRACKS PER SOURCE
GROUP I
GROUP II
GROUP III
GROUP IV
GROUP V
X ± SE
4
8
12
16
20
24
28
32
TIME IN WEEKS

Fig. 21. Electron diffraction patterns characteristic of plutonium oxide were obtained from the aggregation (arrow) of electron opaque particles averaging 0.2 μm in diameter. The particles are clearly associated with remnants of cell structures resembling phagolysosomes, but the cell type cannot be clearly identified. X33, 500.

Fig. 22. Electron diffraction patterns characteristic of plutonium oxide were also obtained from this aggregation (arrow) of electron dense particles. X33, 500.



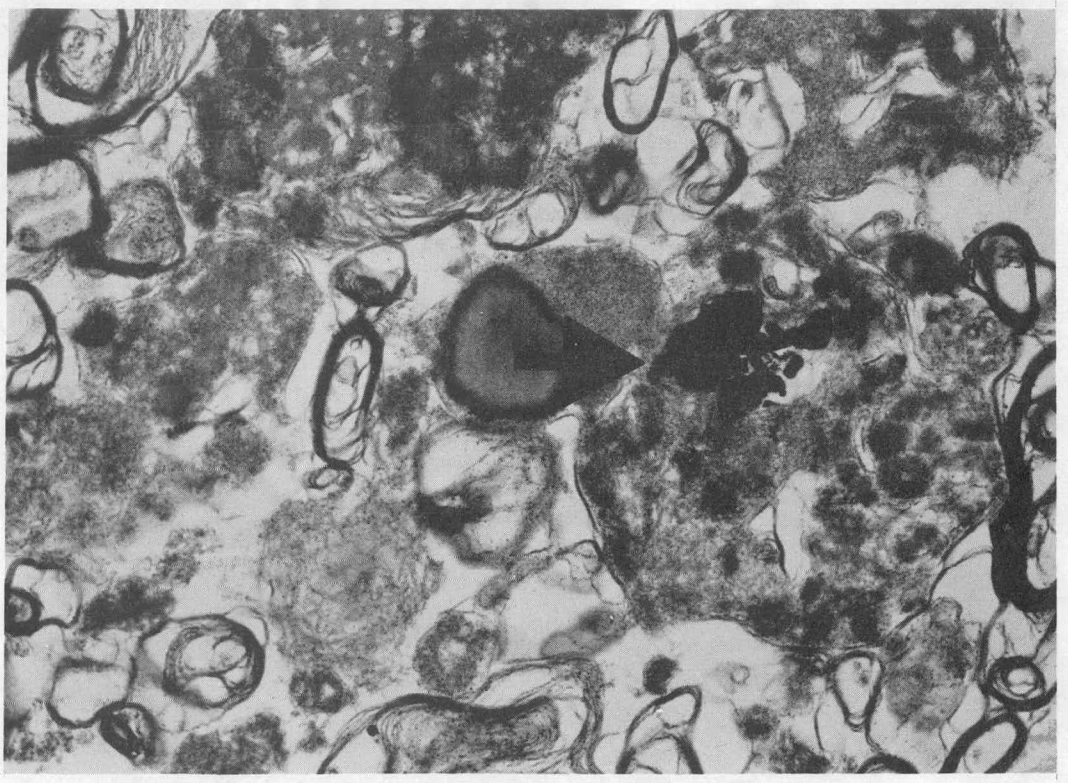
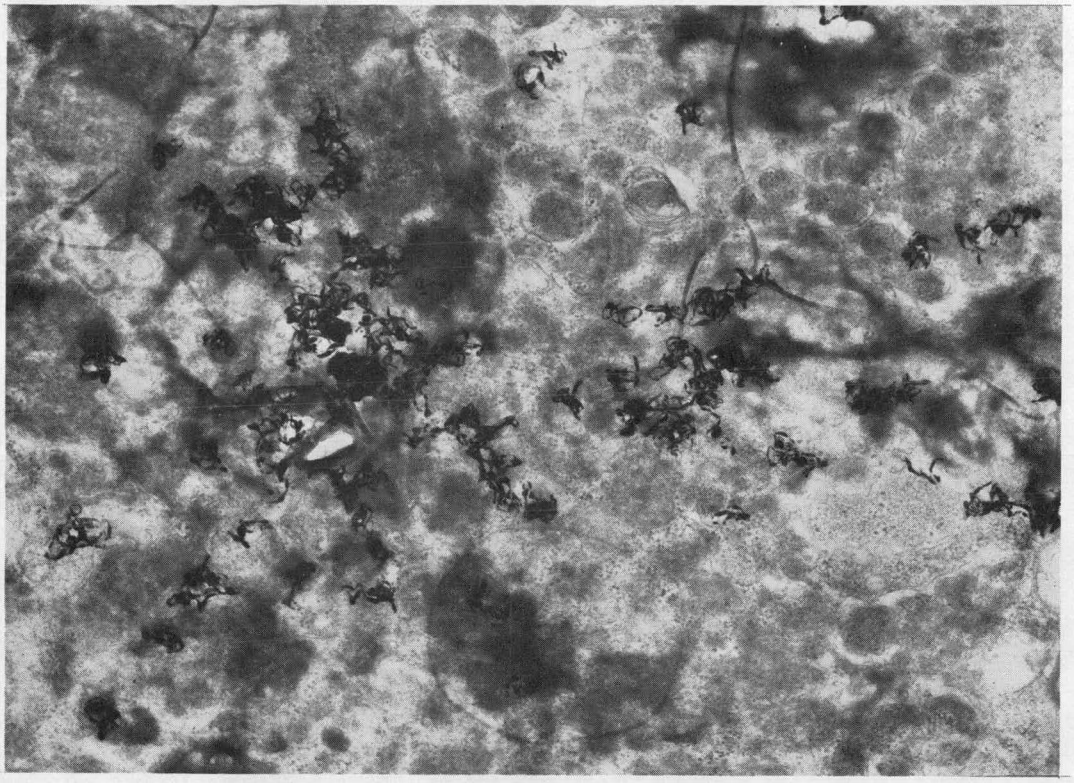


Fig. 23. X22, 750.

These electron microscopic autoradiographs show alpha tracks radiating from plutonium particles. The particles are partially obscured by developed silver grains, but are definitely associated with phagolysosomes.

Fig. 24. X12, 250.



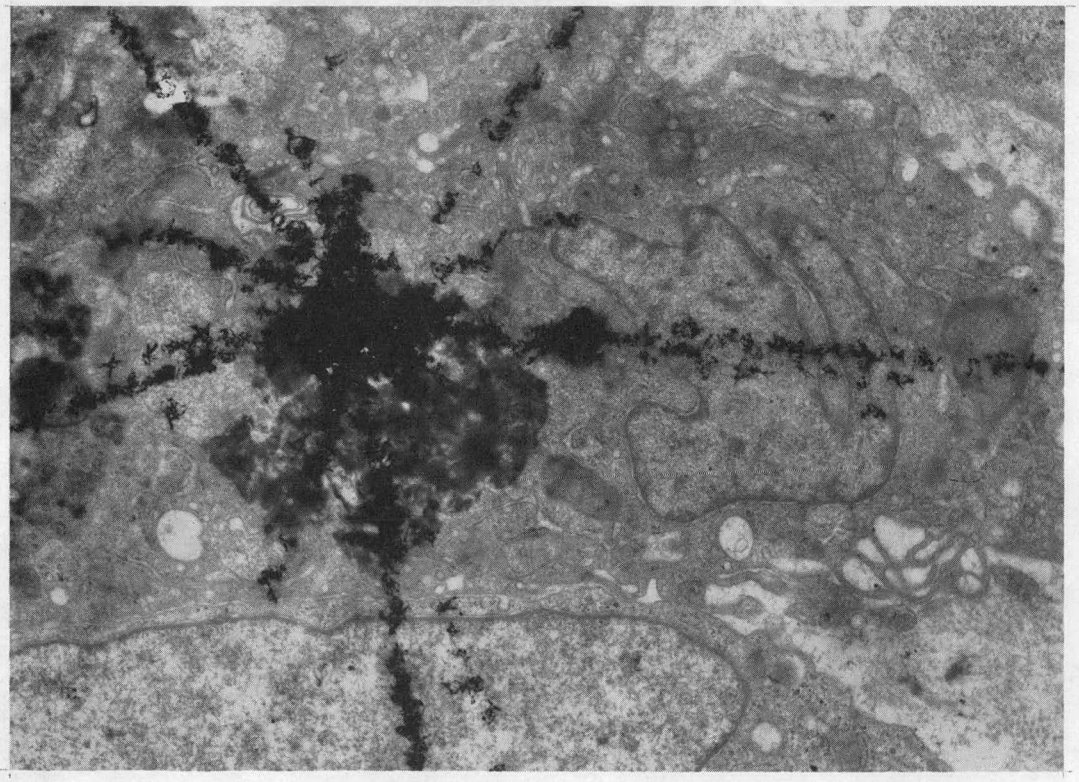


Fig. 25. The cytoplasm of this macrophage contains 2 aggregations of plutonium particles in a phagolysosome. Pronounced degenerative changes are present in the cytoplasm. The plutonium particles range in diameter from 0.03 μm to 0.32 μm. X58, 750.

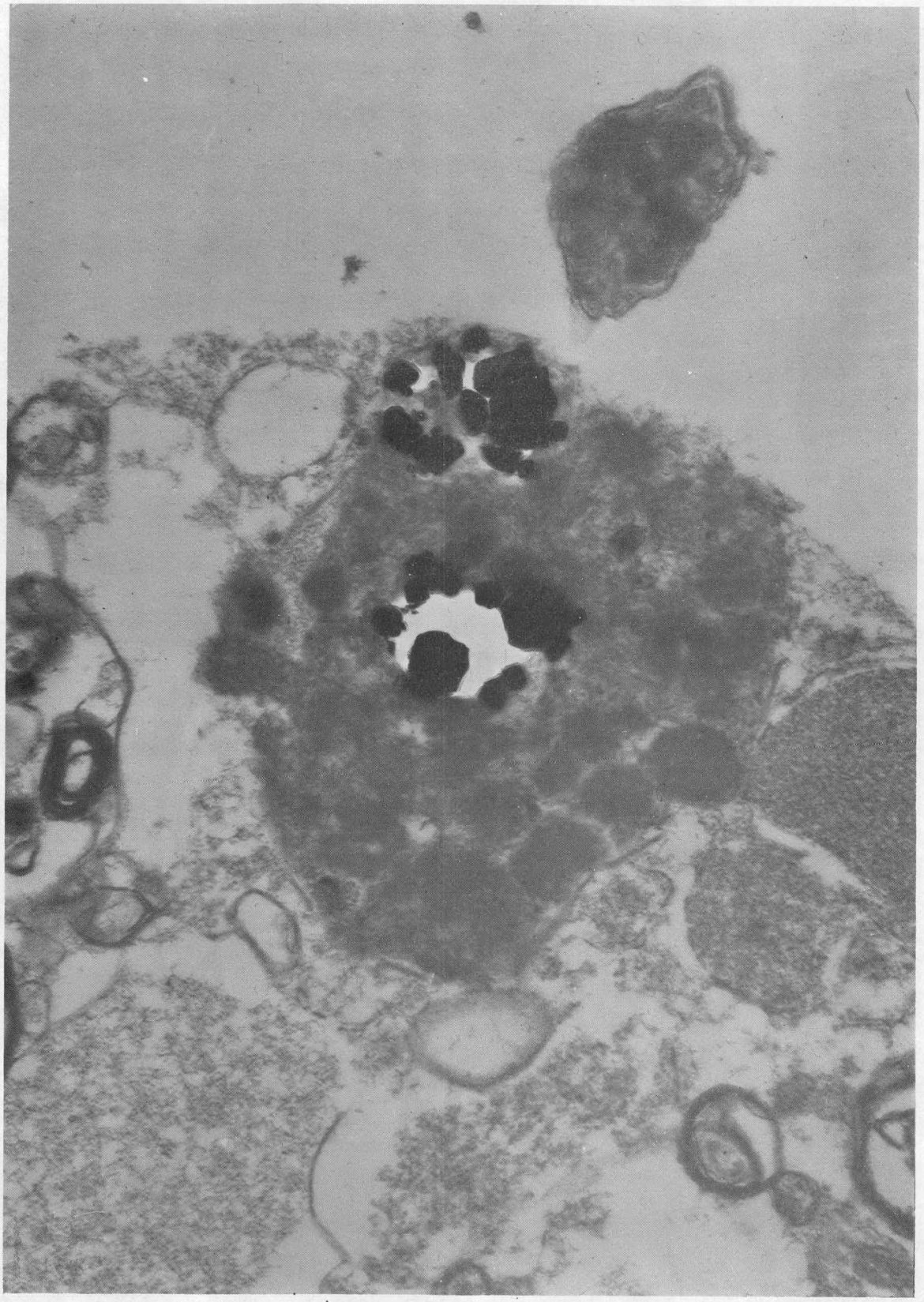


Fig. 26. In this section, the cytoplasm of a macrophage is filled with phagocytosed material. Plutonium particles (arrows) are electron opaque, smooth surfaced, polygonal, and are aggregated in small clear spaces. Adjacent to the plutonium particles, and probably in the same phagolysosome complex, is a moderate amount of granular electron dense material resembling hemosiderin (labeled 1) and electron dense bodies resembling lipofuscin (labeled 2). The plutonium particles average 0.2 μm in diameter. X30, 000.

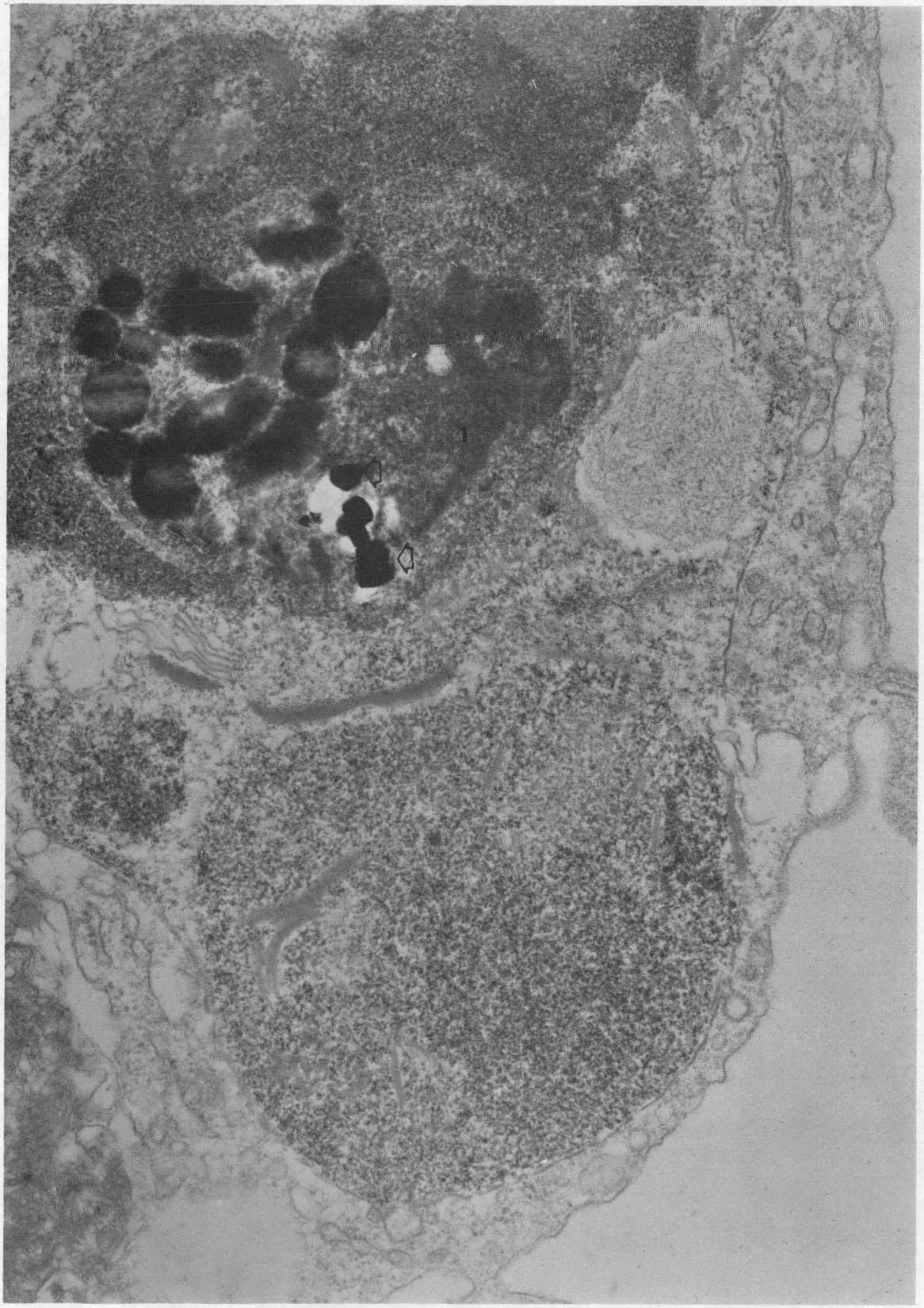


Fig. 27. This field is largely filled with part of a macrophage. The nucleus, mitochondria, and various cytoplasmic organelles are intact. A large phagolysosome complex filling the upper half of the field contains plutonium particles and other electron dense material. The plutonium particles (arrow) are generally electron opaque in the center and partially electron lucent towards the edges. The variation in electron density suggests variations in crystalline structure of the particles. The particles are polygonal, have smooth or serrated edges, and are aggregated in a clear space without a distinct limiting membrane. The larger plutonium particle (arrow) is partially dislocated from the space, probably as a result of displacement during cutting the specimen; this particle measures 1.3 μm in diameter. The adjacent electron dense material resembles hemosiderin and lipofuscin. X30, 000.

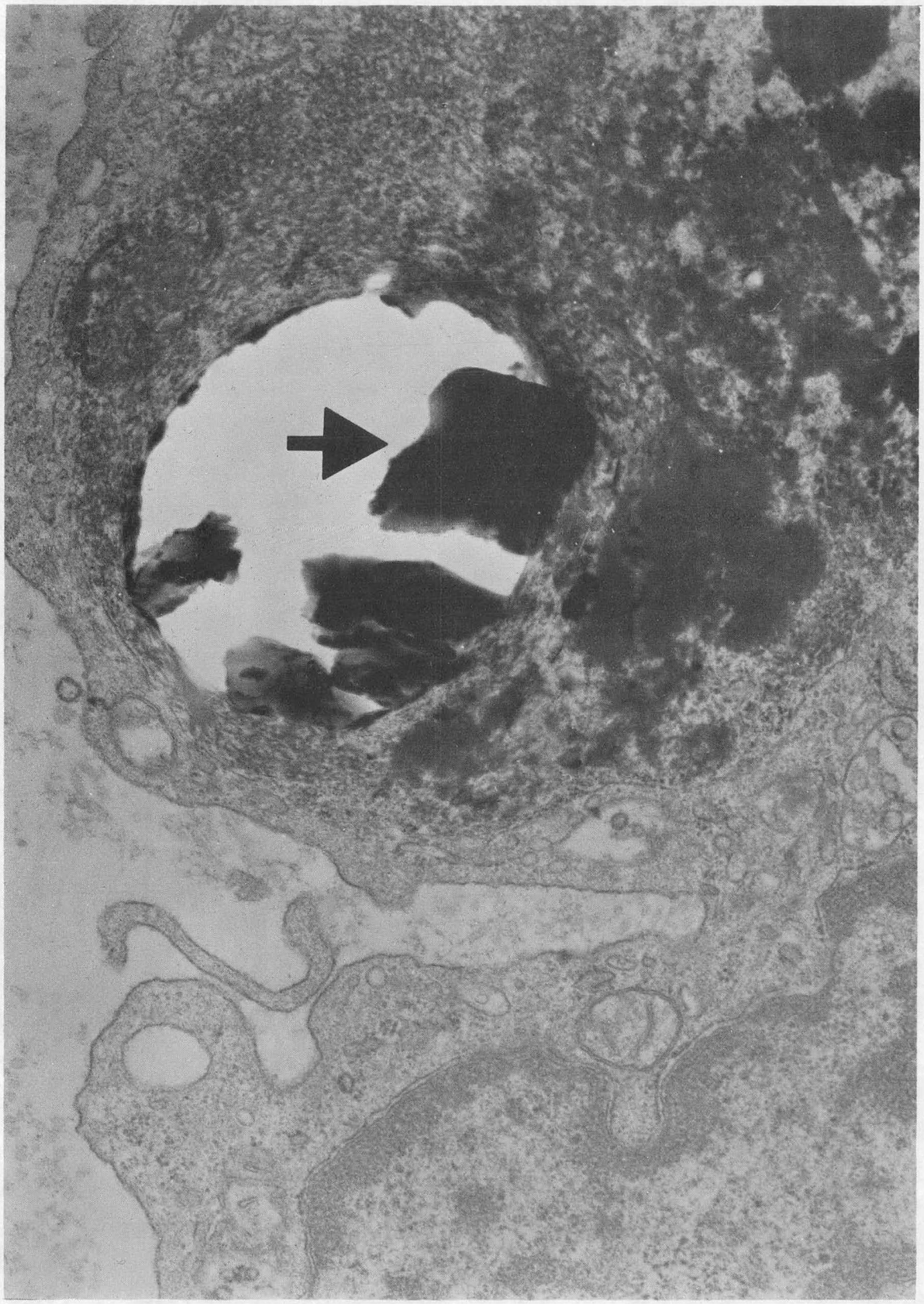


Fig. 28. There are aggregations (arrow) of plutonium particles in remnants of degenerate cells that cannot be clearly identified. The plutonium particles, averaging 0.3 μm in diameter, are electron opaque, polygonal, and are not intimately associated with any membranes. The aggregations are partially surrounded by electron dense structures that probably represent phagolysosomes. X19, 750.

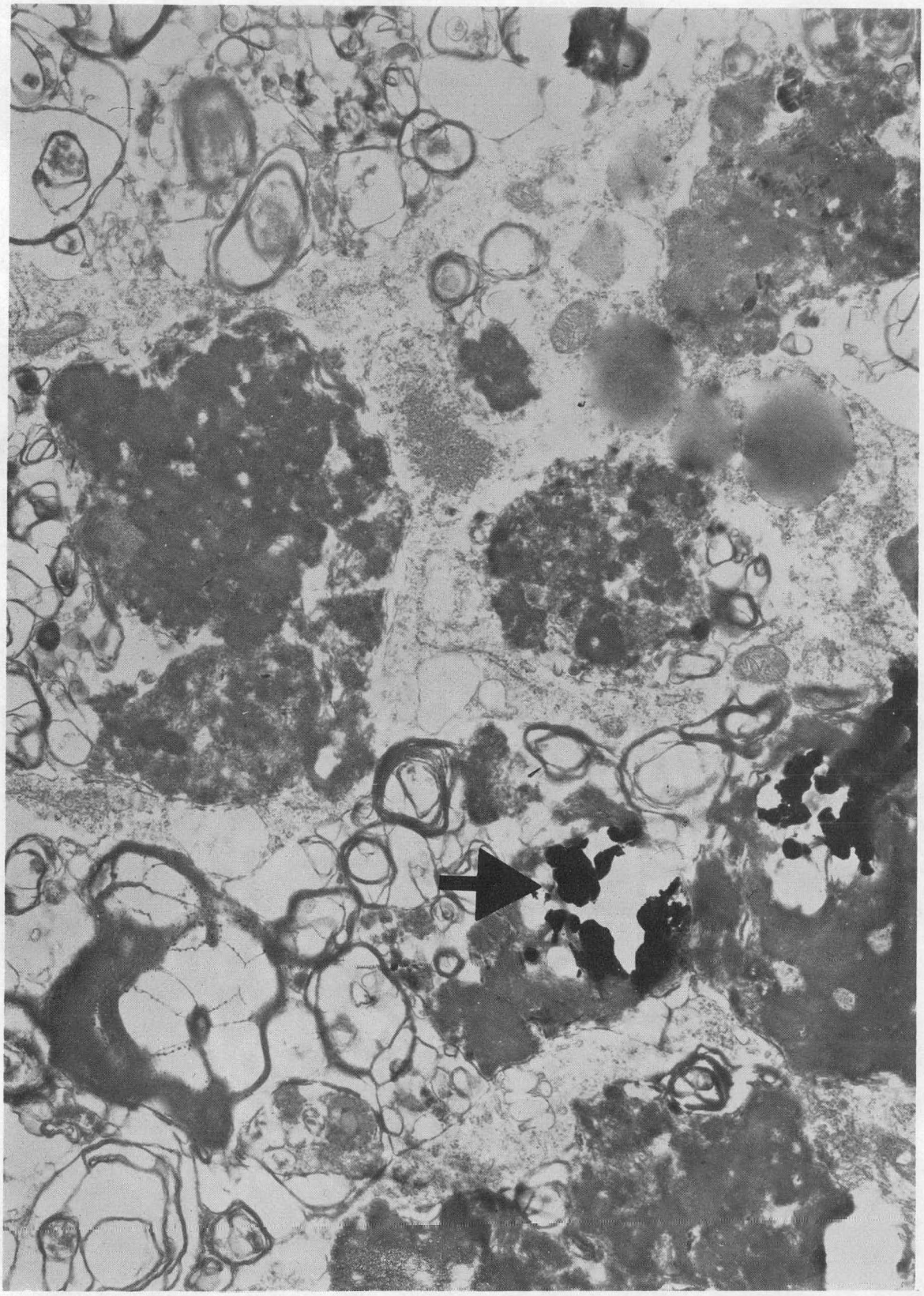


Fig. 29. There are aggregations of plutonium particles (arrows) in a clear space of a degenerate cell. The plutonium particles are polygonal, electron opaque, and are not intimately associated with any membranes. Numerous myelin bodies are present in the cell. The plutonium particles average 0.2 μm in diameter. X19, 750.

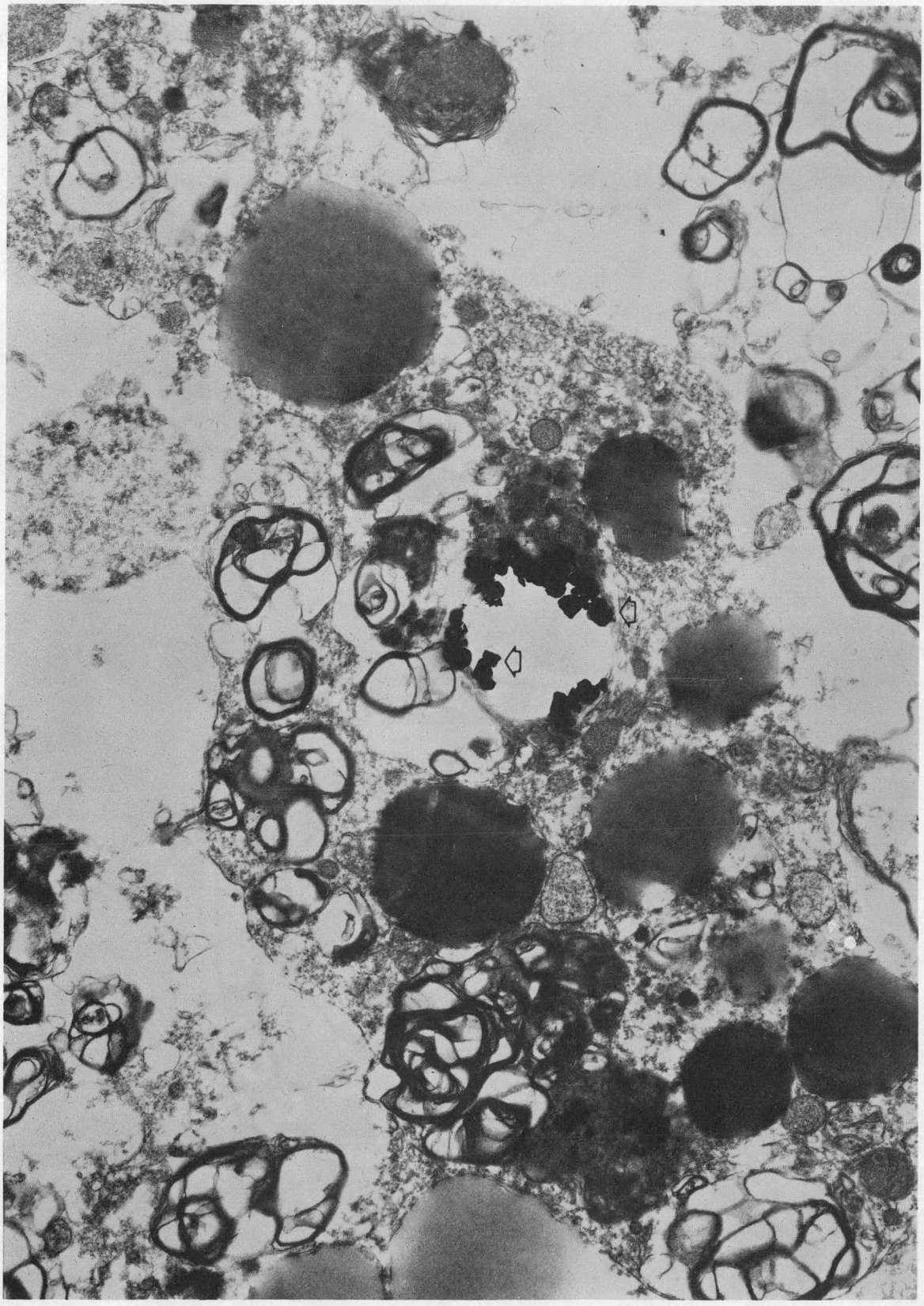


Fig. 30. A large plutonium particle (arrow), measuring 1.2 μm in diameter, is present in a large phagolysosome complex. The cell type cannot be identified from this photomicrograph. The particle is polygonal, has an electron opaque center, and has smooth, slightly serrated, or knobby edges that are less electron dense than the center. The particle is partially dislocated, leaving a clear space. The particle is not intimately surrounded with any membranes. The adjacent electron dense material includes granular material resembling hemosiderin and unidentified small elongated spicules. X30, 000.

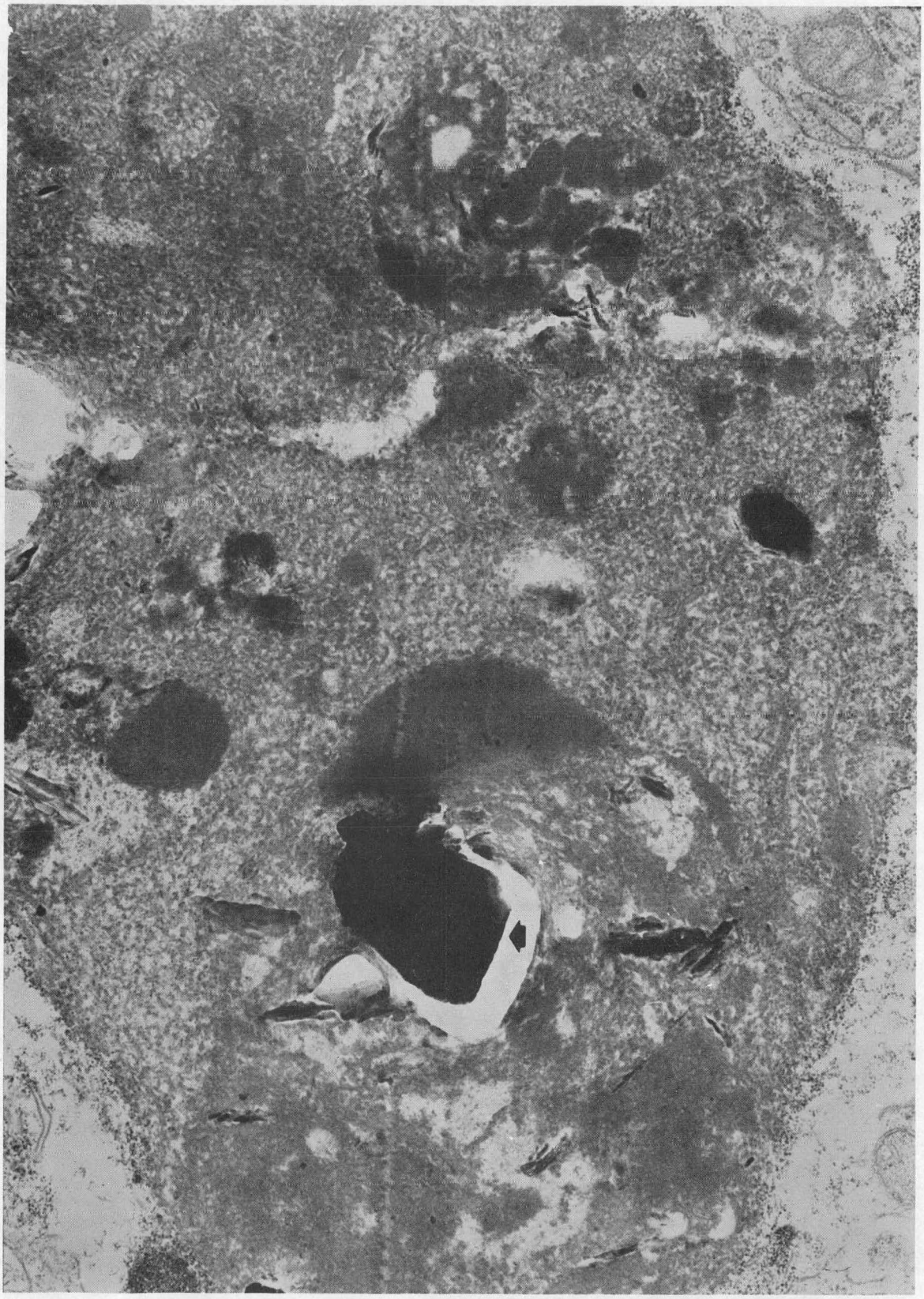


Fig. 31. Plutonium particles (arrow) are aggregated in a clear space in a phagolysosome complex of a degenerate cell. The collagen between degenerate cells probably contributes to the scar tissue observed with the light microscope. The plutonium particles average 0.4 μm in diameter. X13, 750.

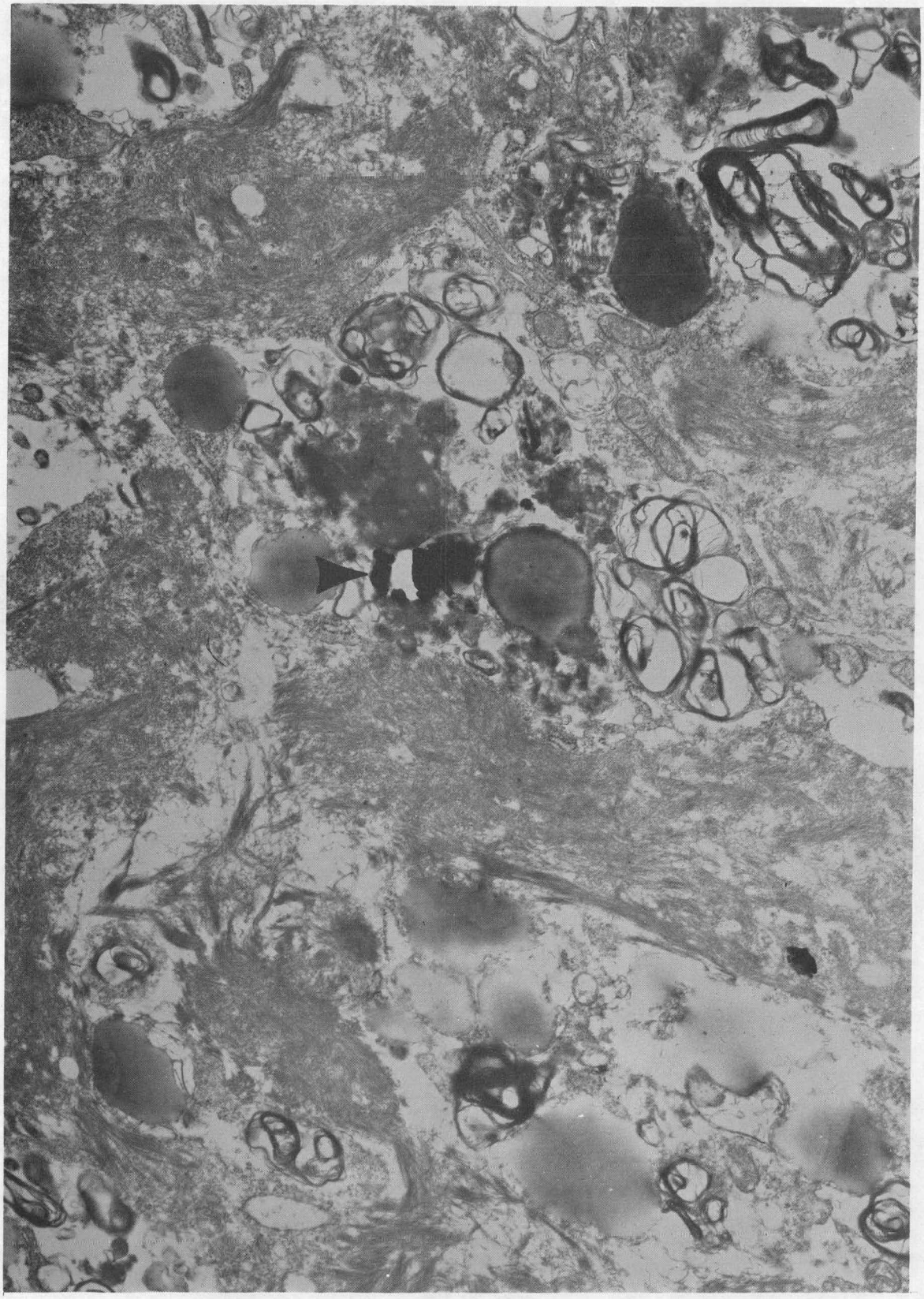


Fig. 32. Plutonium particles (arrows) are electron opaque, polygonal, and are present in clear spaces within a phagolysosome complex of a degenerate cell. The plutonium particles average 0.1 μm in diameter. X30, 500.

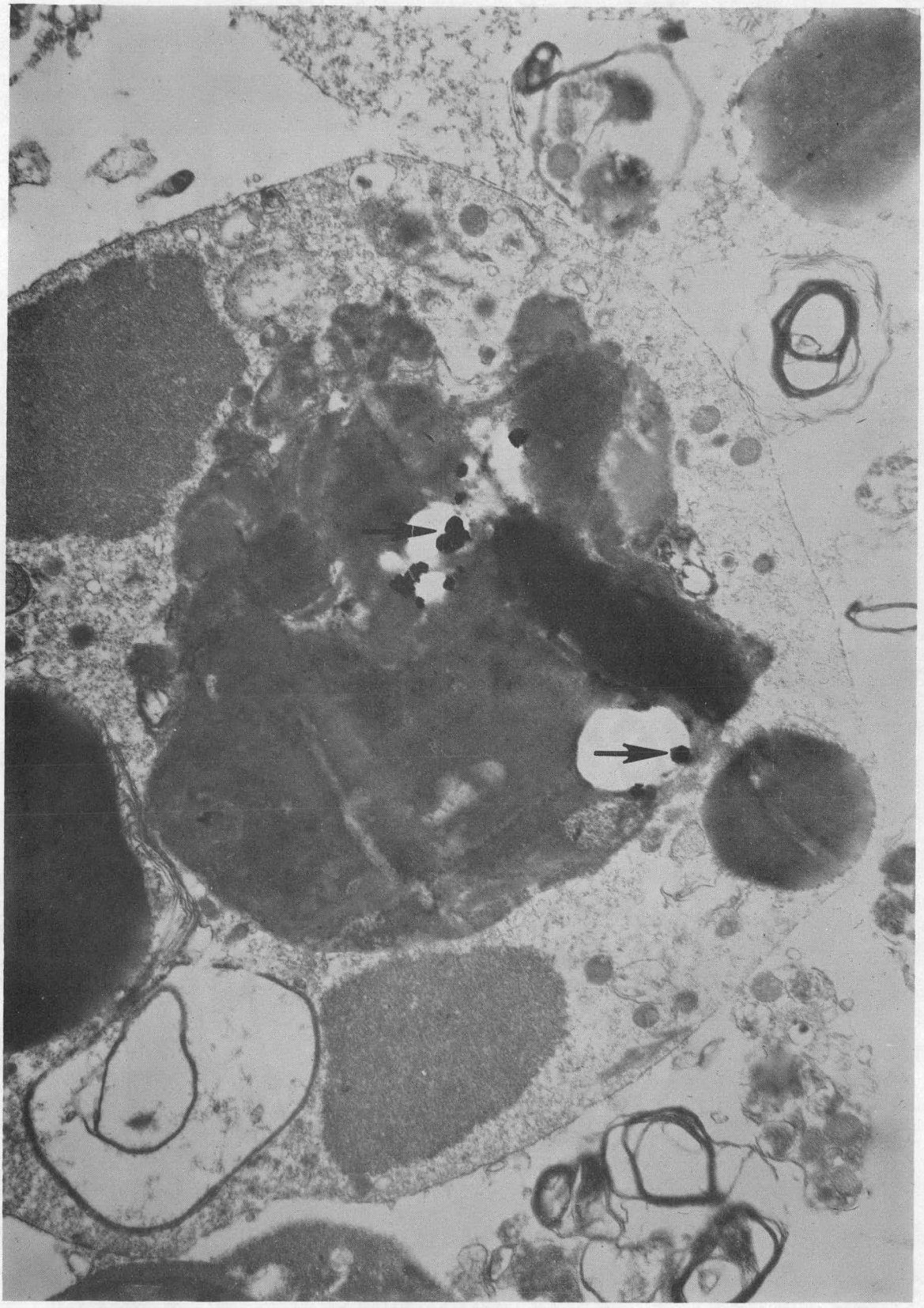


Fig. 33. This specimen is from an area of lymph node heavily damaged by plutonium. Light microscopy showed most of the cells in this area were macrophages, and most of the macrophages contained hemosiderin. The granular electron dense material probably represents hemosiderin phagocytosed in the cytoplasm of the macrophage with the nucleus filling the lower part of the field. X30, 000.

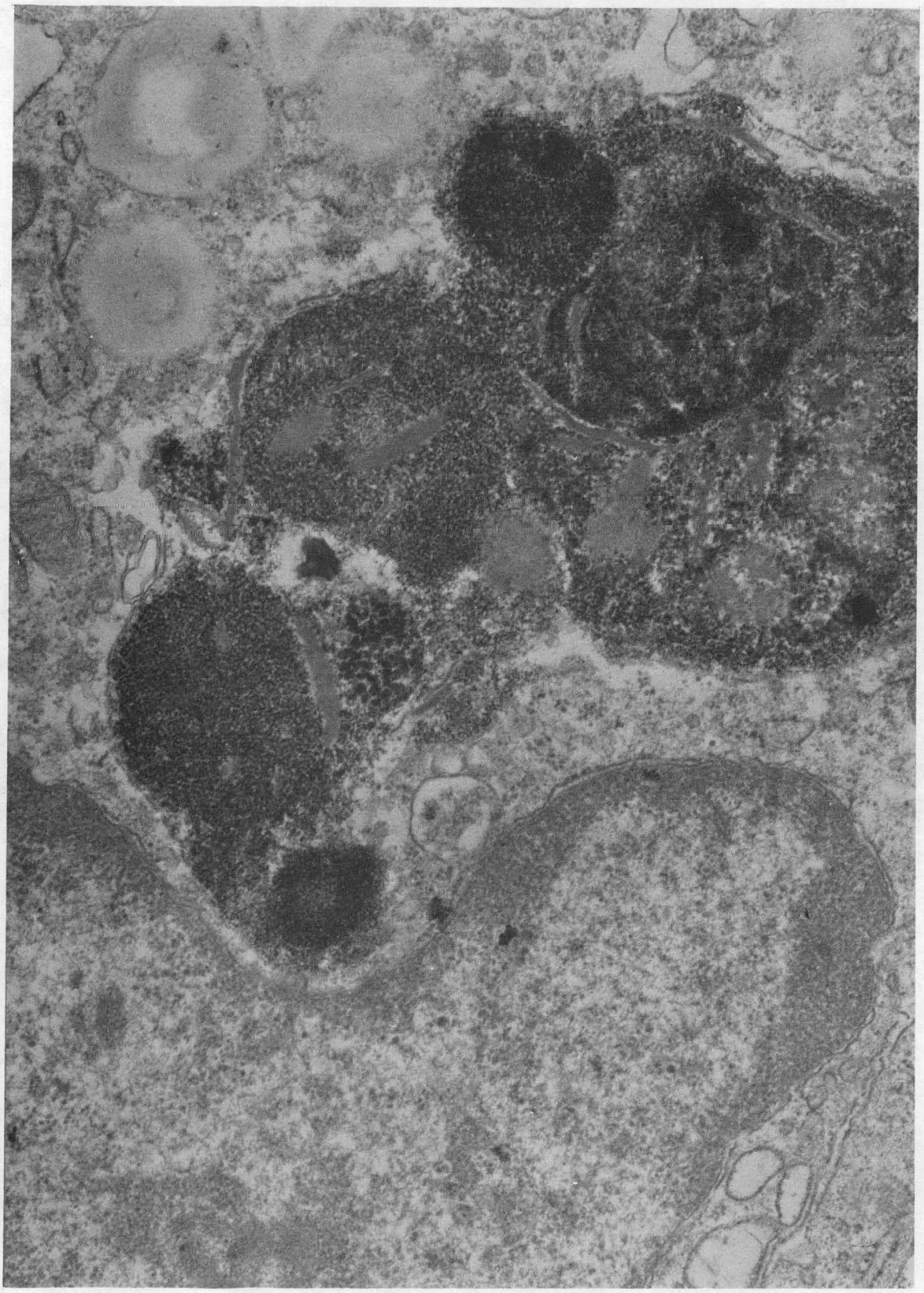
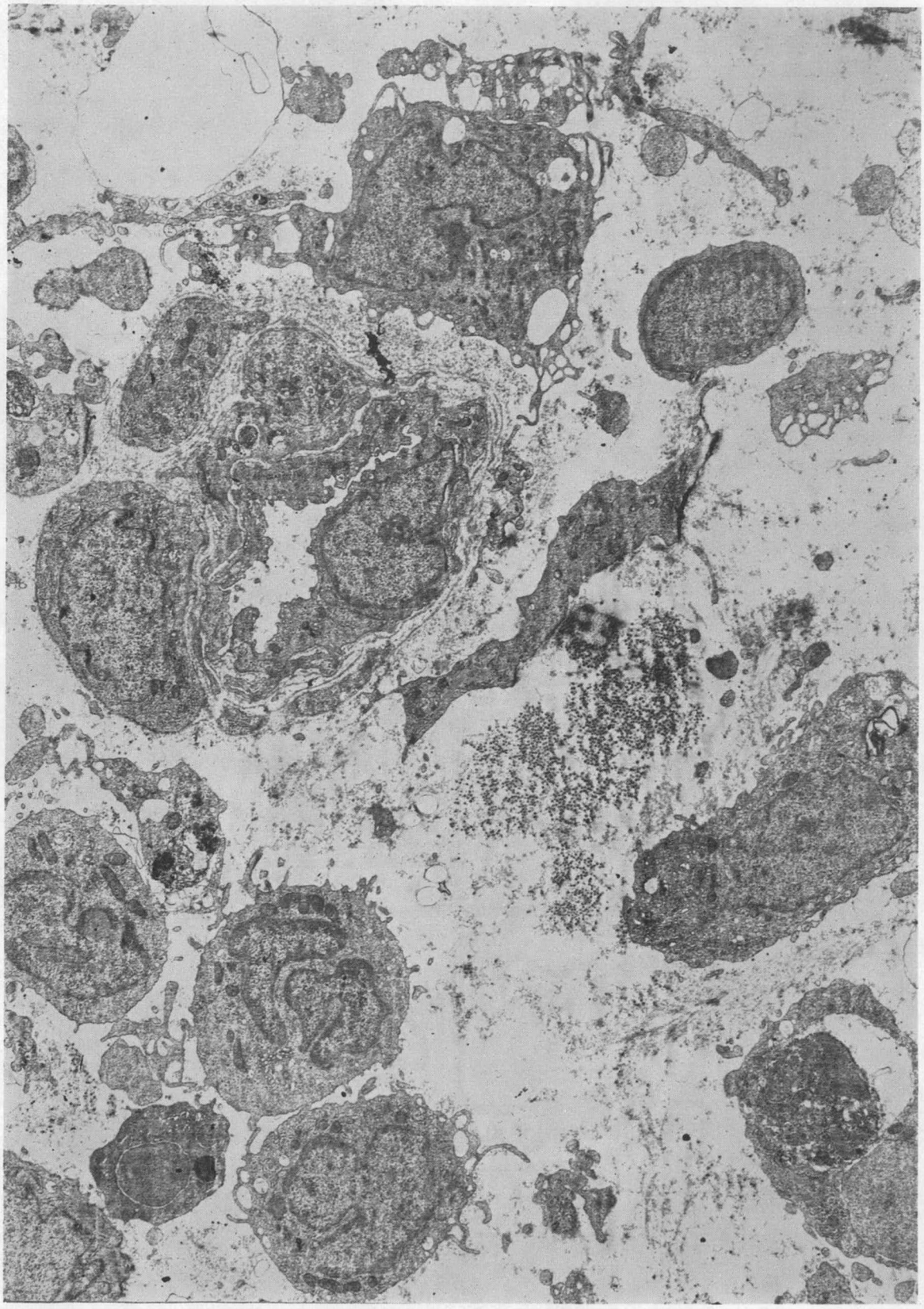


Fig. 34. Plutonium was translocated to this lymph node, causing decreased numbers of cells separated by collagen fibers in an increased amount of intercellular matrix appearing as "empty spaces". The capillary is unremarkable and is surrounded by pericytes. Macrophages have phagocytosed necrotic debris. X3250.



CHAPTER V

DISCUSSION

Translocation Kinetics

The results of this study show that the popliteal lymph nodes are a major deposition site of plutonium oxide injected into the hind paws of beagles. These findings are consistent with the known importance of the superficial cervical lymph nodes in the translocation of plutonium from the front paws of beagles (Johnson, 1969; Bistline, et al, 1972; Gomez, et al, 1972; and Gomez, 1973), and the tracheobronchial lymph nodes in the translocation of plutonium from the lungs of beagles (Bair, 1970; Clark, et al, 1964; and Park, et al, 1962). Since the popliteal lymph nodes are easier to palpate than the superficial cervical lymph nodes and are much more accessible than the tracheobronchial lymph nodes they offer obvious advantages for experimental procedures

Plutonium rapidly accumulated in the popliteal lymph nodes after subcutaneous implantation into the hind paws. Gomez (1973) showed that the afferent lymphatics of the popliteal lymph nodes contained plutonium after subcutaneous implantation. Approximately two-thirds of the plutonium was associated with the cellular fraction of lymph, and one-third was associated with acellular portions of the

lymph. Polyacrylamide gel electrophoresis of the acellular portion of lymph showed that the majority of the plutonium was associated with the beta globulins, including transferrin.

The external iliac lymph nodes accumulate particulates draining from the popliteal lymph nodes. As shown by Drinker, et al, (1934), the popliteal lymph nodes of dogs have a high degree of filtering efficiency. Dogs from Group I (killed 4 weeks post-injection) had relatively little alpha activity in the external iliac lymph nodes as detected by either autoradiography or liquid scintillation. Liquid scintillation did, however, reveal more alpha activity in the left than the right external iliac lymph node, as would be expected with plutonium draining from the left popliteal lymph node. There were dogs from Group II (killed 8 weeks post-injection) that had more alpha activity in the external iliac lymph node than dogs from Group I. Similarly, dogs from Groups III, IV, and V tended to have more alpha activity in external iliac lymph nodes than found in dogs from Groups I and II. There was good correlation between the autoradiographs and liquid scintillation data in each dog except for the right external iliac lymph node from dog 2397. It is likely that the plutonium particles in this lymph node were unequally distributed.

The popliteal lymph node clearance curves were from external (in situ) counting data obtained with a wound counter. Since a significant percentage of the counts obtained with the wound counter were contributed by 17 kev X rays from americium 241, the physical

buildup of americium 241 from plutonium 241 would tend to give higher counts with the wound counter than would be expected from plutonium without americium. Studies by Johnson (1969) with plutonium oxide in the superficial cervical lymph nodes of beagles, however, indicated a preferential translocation of americium over plutonium from lymph nodes. This preferential removal of americium from lymph nodes would tend to counteract the effect of physical buildup of americium on wound counter data.

Other biological factors could also influence wound counter data. The amputation surgery invariably caused edema and mild inflammatory changes that could have caused increased tissue absorption for an undefined length of time. Eventually there was evidence of atrophy on the amputated side that would decrease tissue absorption. Contrasting with the atrophy on the amputated side, there was probably some compensatory hypertrophy in the intact leg. There may even have been behavior factors, i.e., the dogs may have been easier to hold and the positioning of the wound counter probe may have improved as time went on. The relative importance of these biological factors are difficult to assess.

The various influences on the lymph node regression curves, calculated from the wound counter data, were minimized by utilizing counting data for a specific time in dogs from Groups IV (no DTPA) and V (DTPA). At 100 to 210 days there would be less chance of residual surgical effect, less chance of showing effects of atrophy and

hypertrophy, and shorter periods for the effects of the physical build-up of americium or the preferential translocation of americium over plutonium from the lymph nodes. Furthermore, in comparing the regression curves from dogs in Group IV (no DTPA) and Group V (DTPA), the physical and biological factors influencing these curves should be similar between groups of dogs.

Clearance of plutonium from the popliteal lymph nodes was demonstrated in one dog from Group III and after 100 days, for 5 out of the 6 dogs from Groups IV and V. There was variability among dogs, and subcutaneous drainage of plutonium enhanced the clearance of plutonium from the right popliteal lymph node of one dog. These findings are consistent with the clearance of the same reactor grade high-fired plutonium oxide from the superficial cervical lymph nodes of beagles implanted in the forepaw (Bistline, et al, 1972).

There were no significant differences (using Student's t test) between the linear regression curves after 100 days for dogs in Group IV (no DTPA) and Group V (DTPA). These results are compatible with similar findings on studies with the superficial cervical lymph nodes in beagles in which the DTPA did not enhance the removal of air-oxidized plutonium (Johnson, 1969; Johnson, et al, 1972) or $Pu(NO_3)_4$ (Bistline, et al, 1972), and only possibly enhanced the removal of high fired PuO_2 (Bistline, et al, 1972). The action of DTPA is to remove soluble forms of plutonium (Lagerquist, et al, 1965), but

DTPA apparently has little effect in removing particles of plutonium from animals.

Morphological Evaluation

Plutonium was shown to accumulate in the popliteal lymph nodes as phagocytosed particles. Electron micrographs showed plutonium in phagolysosomes of macrophages. Plutonium particles were found only in macrophages. This suggests that, although other cell types may be involved, macrophages would certainly be the most important cell phagocytosing plutonium in lymph nodes. These changes were consistent with the findings of plutonium particles phagocytosed by peritoneal macrophages (Sanders and Adee, 1969) and alveolar macrophages (Adee, et al, 1968; Sanders and Adee, 1970; Lutz, et al, 1970; Nolibe, 1973). This study does not preclude the presence of ionic forms of plutonium in organelles other than phagolysosomes, but the phagocytosed particles would be emitting the great bulk of the radioactivity because of their larger mass.

In autoradiographs plutonium particles are associated with hemosiderin (Taylor, et al, 1972). With the electron microscope, however, plutonium particles in this study were clearly distinct and not enveloped by hemosiderin. This is in contrast to the envelopment of asbestos fibers by hemosiderin to form so-called "asbestos bodies" (Suzuk, et al, 1969). The association of hemosiderin-like material in the phagolysosomes may have been a reflection of phagocytosis of

multiple types of material by the same macrophage. Again, there could have been very small particles of plutonium in hemosiderin that were not detected. Plutonium citrate is associated with the iron transport system in human serum (Stover, et al, 1968); and, the association of plutonium particles to hemosiderin in autoradiographs could be related to a similar transport mechanism between iron and plutonium.

The presence of aggregates of plutonium particles in cells suggests either a breaking down of larger particles with time or a bringing together of smaller particles. Autoradiographs with a time-related increase in the number of tracks per "source" suggests the latter view. Gomez (1973) showed a time-related increase in the number of alpha tracks per star in the superficial cervical lymph nodes of beagles. The fact that the "point sources" in autoradiographs were shown to consist of particle aggregations in electron micrographs demonstrates a disadvantage in sizing plutonium particles with autoradiographs in light microscopic tissue section. The reason for the increasing aggregation of plutonium particles is probably the rephagocytosis of particles after necrosis of cells from radiotoxicity. As with carrageenan granulomata (Spector, 1969), there may be an initial influx of macrophages having a high turnover rate. Later there may be a natural selection of long lived radioresistant macrophages that sequester the insoluble irritants. The long-lived more radioresistant macrophages present after a period of time may have time to

phagocytose more particles. The same number of plutonium particles would have to be phagocytosed by smaller numbers of cells, and this would continue to cause an aggregation of particles.

Necrosis was an early and continued change present in plutonium contaminated lymph nodes. Common features were the scattered distribution of single necrotic cells between viable cells and the necrosis of occasional cells away from the proximity of plutonium particles. This patchy distribution of necrosis in lymph nodes has been seen with other types of irradiation (Christopherson, 1955). The scattered distribution of the necrosis also resembled apoptosis as described by Kerr (1972). Apoptosis, or abiosis, is single cell necrosis that seems to occur physiologically to balance mitosis.

The single cell nature of the necrosis was compatible with the direct action of irradiation. There were no areas of coagulative necrosis or indications of infarction. The vascular lesions were in areas severely damaged and were probably sequelae rather than primary causes of necrosis. Engeset (1964) demonstrated lymph node atrophy in rats due to vascular lesions after total body irradiation, but the local continuous irradiation from plutonium would cause continuous damage and continuous direct necrosis of cells.

Coincident with necrosis in the pathogenesis of plutonium injury to lymph nodes was an increase in the number of reticular cells. It is probable that some prominence of reticular cells in histologic sections was apparent merely because of decreased numbers of

lymphoid cells from necrosis and the unmasking of reticular cells. However, in the time periods of the study, over 3 weeks before the first necropsies, there was definite hyperplasia of reticular cells as evidenced by increased numbers of reticular cells and mitotic figures. The increased numbers of reticular cells were accompanied by increased numbers of macrophages that phagocytosed the plutonium particles. There were miscellaneous additional infiltrating inflammatory cells, primarily neutrophils, that were diffusely distributed and probably responding to non-specific injury. These proliferative and exudative changes probably caused the increased weights of popliteal lymph nodes in dogs from Group I.

Fibrocytic proliferation was observed with reticular cell hyperplasia in dogs from Group I and fibroplasia generally became the most prominent lesion in the dogs from the remaining groups. Even though reticular cells are radioresistant, high amounts of radiation energy as delivered by plutonium particles eventually causes necrosis of these cells (Holsten, 1970). Fibrogenesis is a general response to necrotic cells, perhaps through the release of phospholipids by the necrotic cells (Heppleston, 1967). The end stage was a smaller lymph node with a lesion characterized as scar tissue with few admixed cells. Finally, cavitation occurred probably as a result of the high energy coming from the plutonium particles causing a direct effect on the breakdown of collagen fibers. The direct breakdown of collagen fibers has been described in man as being due to the high

energy of plutonium particles, and this was contrasted to the softening of thorium induced granulomas caused by the chronic effect of radon exposure on blood vessels (Lushbaugh, et al, 1967).

The eventual sequestering of plutonium particles in the scar tissue of lymph nodes probably alleviates the potential of the alpha radiation damaging the host. The alpha particles only penetrate soft tissues up to 50 μm, and the presence of rather hypocellular scar tissue for this distance around the plutonium means that the alpha particles have ceased to come in contact with parenchymal cells. If parenchymal cells are no longer damaged directly by irradiation, there may be less chance of mutagenic or other action causing tumor induction.

The pronounced scarring of the popliteal lymph nodes may, however, alter the filtering and immunologic functions of these lymph nodes. Decreased filtering capacity of popliteal lymph nodes of rats was noted after localized X-irradiation (Sinha, et al, 1970). Brightwell, et al, (1973) tentatively attributed the observed inhibition of urethane-induced pulmonary adenomas by inhalation of plutonium to alpha irradiation interferring with the immuno-depressive effect of urethane.

CHAPTER VI

SUMMARY

The lymph node clearance of plutonium oxide subcutaneously implanted into the hind paws of beagles was studied. External in situ scintillation data were collected from the popliteal lymph nodes of each dog. The left hind paw was amputated 4 weeks after implantation to prevent continued deposition of plutonium oxide particles in the left popliteal lymph node. Groups of beagles were sacrificed 4, 8, 16, and 32 weeks after implantation for histopathologic, electron microscopic, and radiochemical analysis of regional lymph nodes.

Plutonium rapidly accumulated in the popliteal lymph nodes. Histopathologic changes in the popliteal lymph nodes with plutonium particles were characterized primarily by reticular cell hyperplasia, increased numbers of macrophages, necrosis, and fibroplasia. Eventually the plutonium particles became sequestered by scar tissue that often replaced the entire architecture of the lymph node. Light microscopic autoradiographs of the popliteal lymph nodes showed a time related increase in number of alpha tracks per plutonium source. Electron microscopy showed that plutonium particles were aggregated in phagolysosomes of macrophages.

There was slight clearance of plutonium from the popliteal lymph nodes of dogs monitored for 32 weeks. The clearance of plutonium particles from the popliteal lymph nodes was associated with necrosis of macrophages. The external iliac lymph nodes contained fewer plutonium particles and had less severe histopathologic changes than the popliteal lymph nodes. The superficial inguinal lymph nodes of one dog contained appreciable amounts of plutonium. Treatment with diethylenetriaminepentaacetic acid (DTPA) did not influence the clearance of plutonium from the popliteal lymph nodes.

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