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LYMPH TRANSPORT OF $^{239}\text{PuO}_2$ IN DOGS

By

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MASTER

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ABSTRACT OF THESIS

LYMPH TRANSPORT OF $^{239}\text{PuO}_2$ IN DOGS

The translocation of high-fired $^{239}\text{PuO}_2$ via the lymphatic system from a simulated puncture wound in a dog's paw was studied with two major objectives in mind. The first was to study the effect of lymphadenectomy on PuO_2 translocation, and the second was to determine the mode by which PuO_2 is rapidly translocated from wound site to lymph node. Minor experiments were performed to clarify anatomical questions which arose during this study.

The lymphadenectomy experiments included lymph node excisions on the day of Pu implant, as well as excisions 14 days after implant. Comparisons were made between dogs with and without excision of the left superficial cervical lymph node (LSCLN). Build-up of Pu in the LSCLN was determined by in vivo counting of the low-energy x-ray complex with a thin NaI(Tl) detector. The dogs were killed at selected times after implant and the α -activity was determined in selected tissues.

Higher levels of Pu were found in the liver, spleen and hepatic lymph nodes of lymphadenectomized dogs than in the intact dogs. Implications of the data to therapeutic lymph node removal from workers contaminated with Pu are discussed.

The data from disc electrophoresis of lymph and autoradiographic data from packed lymph cells and lymph nodes suggest that PuO_2 is primarily transported by the cellular fraction of lymph. The PuO_2 transported in the acellular fraction was mainly associated with the β -globulin protein fraction, which includes transferrin, the major Pu-transport protein in plasma. The data also suggest that the rapid PuO_2 transport was associated with the protein-bound Pu; the slower transport of large PuO_2 particles was via the cellular fraction of lymph.

The minor, anatomical studies provided information concerning size and number of lymph nodes, as well as providing information concerning the translocation routes of Pu from the paw to either the LSCLN or the left axillary lymph node. The latter experiment clarified Pu deposition results which had not been explained in earlier experiments at this laboratory.

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CHAPTER I

INTRODUCTION

According to Eisenbud (1967), man's knowledge of radioactive materials until 1939 was due to natural sources alone. Some of these naturally occurring radionuclides were commercially used, and during the first two or three decades following the discovery of radioactivity in the late 1800's, about two pounds of radium were extracted from the earth's crust. Misuse of this small amount of radium resulted in the death of at least 100 people (Eisenbud, 1967). In comparison with the much greater quantities of radioactive materials that became available after 1942, the earlier years of this century provided relatively few opportunities for exposure to radioactive substances. The pre-World War II experience was nevertheless grim.

With the expanding use of plutonium for peaceful as well as defense purposes, the opportunities for accidental deposition within the body are expected to increase (Tremmel, 1968 and Roddis, 1969). Based on animal studies, plutonium-239 (^{239}Pu) is considered to be one of the most toxic materials known to man (Norwood, 1972 a). The hazard is due to alpha emissions from internally deposited ^{239}Pu , which has a very long effective half-life. Assessment of the deposition

and need for therapy is possible only if the metabolism of ^{239}Pu within the body is understood.

Maximum information must be obtained by subjecting a variety of laboratory animals to the effects of various chemical forms of ^{239}Pu administered via all possible routes of entry (Norwood, 1972 b). For the past few years, researchers in the Department of Radiology and Radiation Biology at Colorado State University have studied the movement of plutonium and americium for subcutaneous implants which were simulants for puncture wounds. These studies were initiated because of the lack of data concerning plutonium and americium translocation via the lymphatic system.

The purposes of the present study were twofold:

1. To study the effect of lymph node removal (lymphadenectomy) on the systemic translocation of high-fired $^{239}\text{PuO}_2$ from the implant site in a dog's paw.
2. To gain new knowledge about the mechanism involved in the rapid translocation rate of $^{239}\text{PuO}_2$ from the implant site to the superficial cervical lymph node, the first major node draining the implant site.

CHAPTER II

REVIEW OF THE LITERATURE

Some Characteristics of Plutonium

Plutonium (Pu) was the second transuranium element to be discovered. In 1940 Glenn T. Seaborg et al. succeeded in preparing a new isotope of neptunium ($^{238}_{93}\text{Np}$) which decayed to $^{238}_{94}\text{Pu}$ by bombarding uranium with deuterons, heavy isotopes of hydrogen, in the 60-inch cyclotron at Berkeley, California (Seaborg, 1963):

There are now 15 known isotopes of Pu with atomic weights from 232 to 246, inclusive. These isotopes are listed in Table 1. The most important isotope of plutonium is $^{239}_{94}\text{Pu}$ ($T_{1/2} = 24,360 \text{ y}$), produced in large quantities in nuclear reactors from natural uranium (Weast, 1964):

Plutonium is a member of the actinide series and its chemistry is qualitatively similar to that of the rare earth elements. It is a highly reactive metal, combining readily with oxygen. The oxides are relatively insoluble, as are the fluorides and hydroxides. Plutonium has four oxidation or valence states (3, 4, 5 and 6) and forms

TABLE 1
Radioactive Decay Properties of Plutonium
Seaborg, (1963)

Plutonium Isotope	Half-Life	Mode of Disintegration	Energy (MeV)
^{232}Pu	36 m	E. C. ($\sim 90\%$), α ($\sim 10\%$)	6.58
^{233}Pu	20 m	E. C. ($>99\%$), α (0.1%)	6.30
^{234}Pu	9.0 h	E. C. (94%), α (6%)	6.196, 6.145, 6.025
^{235}Pu	26 m	E. C. ($>99\%$), α ($3 \times 10^{-8}\%$)	5.85
^{236}Pu	2.85 y	α β stable	5.763, 5.716, 5.610, 5.448
$^{237\text{m}}\text{Pu}$	0.18 s	I. T.	0.145
^{237}Pu	45.6 d	E. C. ($>99\%$), α ($3.3 \times 10^{-3}\%$)	5.65, 5.36
^{238}Pu	86.4 y	α β stable	5.491, 5.452, 5.352
$^{239\text{m}2}\text{Pu}$	1.93×10^{-7} s	I. T.	0.392
$^{239\text{m}1}\text{Pu}$	1.1×10^{-9} s	I. T.	0.286

Table 1 Continued

Plutonium Isotope	Half-Life	Mode of Disintegration	Energy (MeV)
^{239}Pu	24,360 y	α β stable	5.147, 5.134 plus others ranging from 5.10 to 4.92
^{240}Pu	6,580 y	α β stable	5.159, 5.115 plus others ranging from 5.01 to 4.85
^{241}Pu	13.2 y	β^- (>99%) α ($5 \times 10^{-3}\%$)	0.0205 4.890, 4.845
^{242}Pu	3.79×10^5 y	α β stable	4.898, 4.854
^{243}Pu	4.98 h	β^-	0.58, 0.49
^{244}Pu	7.6×10^7 y	α	4.55 (estimated)
^{245}Pu	10.6 h	β^-	
^{246}Pu	10.85 d	β^-	0.33, 0.15

quite stable compounds with all the non-metallic elements except the rare gases (Norwood, 1972 a). It also exhibits four ionic valence states in aqueous solutions: Pu^{+3} (blue-lavender), Pu^{+4} (yellow-brown), PuO_2^{+} (presumably pink) and PuO_2^{+2} (pink-orange). The ion PuO_2^{+} is unstable in aqueous solutions, disproportionating into Pu^{+4} and PuO_2^{+2} ; the Pu^{+4} thus formed, however, oxidizes the PuO_2^{+} into PuO_2^{+2} , itself being reduced to Pu^{+3} , giving finally Pu^{+3} and PuO_2^{+2} . Plutonium forms binary compounds with oxygen (PuO_2 and intermediate oxides of variable composition); with the halides (PuF_3 , PuF_4 , PuCl_3 , PuI_3); with carbon, nitrogen and silicon (PuC , PuN , PuSi_2); in addition, oxyhalides are well known (PuOCl , PuOBr , PuOI) (Weast, 1964). Plutonium melts at 640°C and boils at 3327°C .

A simplified decay scheme for ^{239}Pu with the three major alpha disintegrations is shown:

(Radiological Health Handbook, 1960)

Ten different alpha particles of ^{239}Pu have been identified, but only three of these contribute significantly to dose. These have energies of 4.145 MeV, 72.5%; 5.134 MeV, 16.8%, and 5.096 MeV, 10.7%, respectively (Snyder, 1967). The emitted alpha particles have an average energy of 5.15 MeV and a range of about 40 microns in water and soft tissue and about 24 microns in bone (Mays and Sears, 1962; Norwood, 1972 a). Plutonium-239 also emits a few weak x-rays with an average energy of 17 keV. One microgram of ^{239}Pu is equivalent to 0.064 microcuries or about 1.4×10^5 alpha disintegrations per minute (Langham, 1959).

Plutonium-239 has assumed a dominant position among the transuranium elements because of its successful use as a fissionable ingredient in nuclear weapons and as a key material in the development of nuclear power (Weast, 1964). It was pointed out by Cleveland (1967) that plutonium dioxide is the most important of all Pu compounds. This stems from its desirable properties, such as high melting point, irradiation stability, compatibility with other metals and ease of preparation. These properties make it one of the most promising compounds for use as a nuclear fuel. The industrial hazard of PuO_2 is reflected in a report by Hammond and Putzier (1964) which states that "a major portion of the accidental exposures to plutonium occurs with the oxide or metal form at processing facilities such as the Rocky Flats Plant of the Dow Chemical Company."

Plutonium Hazards in Industry

Contaminated wound cases from Pu handling facilities were apparently not reported in the open literature prior to 1958. Langham et al. (1962) and Langham (1972) discussed the management of potentially contaminated wounds and recounted the problems and cases encountered during the World War II nuclear weapon building period. In 1962 the literature contained reports of many of the hazards associated with Pu contamination (Cable, 1962; Durbin, 1962; Bair et al.; Herring et al.; Norwood, 1962; Evans, 1962; Swanberg, 1962 and Roesch and Palmer, 1962). In that same year, however, Glasstone (1962) reported the following:

"As in the case with fallout particles, significant entry of plutonium and uranium oxides into the body can occur through the respiratory tract; even then, the nose and lungs act as effective filters. Because of their small solubility, absorption of the oxides by the gastrointestinal system is very low. Furthermore, penetration of the intact skin is impossible and although the material may enter where the skin is broken, it will be localized at the point of access. It may then be cleansed away at some later time, without appreciable translocation to the blood stream or other body tissues."

Unfortunately, Pu contamination is not restricted to the point of access. Relatively complete reviews of the literature regarding the modes of entry, distribution, excretion and means of evaluating plutonium within living system have been published (Thompson, 1967; Wilson, 1967; Durbin, 1971; Stover and Jee, 1972; Thompson and Bair, 1972). Thompson (1967) has classified Pu exposure hazards as

follows: (1) the hazard of Pu distribution within the body by way of the blood stream, (b) the hazard of Pu deposition in the lungs and associated lymph nodes, (c) the hazard of Pu deposited beneath the skin or in muscle in a relatively nonabsorbable form as a result of contaminated puncture wounds. These hazards are interrelated in many cases. For example, in a contaminated puncture wound, both hazards (a) and (c) may have a mutual bearing on the internal distribution of the activity.

In industry Pu almost always enters the body by inhalation or via contaminated wounds. Absorption via the gastrointestinal tract or intact skin is so small as to be negligible under normal conditions (Norwood, 1972 a, b). Inhalation is the most common route of entry, except at production plants such as the Rocky Flats Plant of the Dow Chemical Company, where the hazard of contaminated wounds is great because of intimate handling of the unfinished metal (Norwood, 1972 a).

The criteria which might be used to measure the hazard of a substance such as Pu would relegate it to the category of greatest potential hazard. Injury statistics indicate otherwise, for there have been, to this time, no serious injuries reported in workers due to the deposition of Pu (Thompson, 1967; Norwood, 1972 a). However, a lesion described as probably precancerous, resulting from a relatively small subcutaneous deposition of Pu in a wound, has been reported (Lushbaugh and Langham, 1962).

Radiological health aspects of the chemical and metallurgical processing of Pu require that a considerable portion of the work be done in glove boxes or other such containment systems. Experience at Rocky Flats has shown that although effective exposure control methods have been successfully employed in Pu processing, the potential for contamination from wounds is significant (Hammond and Putzier, 1964). Puncture wounds occur most frequently on the fingers or hands from sharp objects or broken glass in a glove box (Wilson, 1967).

Contaminated wound management has been difficult due to the following variables: different physical and chemical forms, unknown depth of deposition, type of tissue involved, amount of dispersion of Pu in the wound, particle sizes involved and variance of individual response (Langham et al., 1962; Norwood, 1962).

Internal Exposure Evaluation

As a basis for health protection work it was necessary to establish the maximum permissible body burden for plutonium. At first a tentative limit was established on an arbitrary basis. Russell and Nickson (1951) stated that from purely physical considerations it seemed that Pu, weight for weight, should be approximately one-fiftieth as toxic as radium. Since the tolerance amount of radium in the body is generally accepted as 0.1 micrograms, the body burden for plutonium was initially set at 5.0 micrograms. Later, on the

basis of experimental evidence, the maximum permissible body burden of Pu was lowered to its present limit of 0.6 micrograms (Langham et al., 1962).

Because of the potential hazard, it was essential to devise a means of detecting and measuring quantities of Pu in an individual which were less than the maximum permissible body burden. At first it appeared that this could be achieved by the analysis of excreta (Russell and Nickson, 1951).

In order to estimate the Pu content of the body by excreta analysis, it was necessary to determine the rate of Pu excretion. Two widely used methods for estimating Pu deposition are those of Langham (1956), which relates body burden and urinary excretion of Pu, and Healy (1957), which relates lung burden and urinary excretion of Pu.

Reports describing the uncertainties in calculating Pu body burdens from urine analysis have been published (Snyder, 1962; Hammond and Putzier, 1964; Beach et al., 1966; Jech et al., 1972). Nevertheless, nuclear facilities such as Rocky Flats routinely analyze employee urine specimens for Pu and Am (Lagerquist et al., 1972), because in many cases excreta analysis may be the only indicator of the amount of Pu present.

Development of radiation detectors has allowed in situ measurements following suspected exposures and has aided in making assessments of Pu body burdens in industrial situations. Sodium iodide

[NaI(Tl)] detectors have the capability of measuring less than a maximum permissible liver or lung burden (Boss and Mann, 1967). In vivo evaluation of deposition and translocation of ^{239}Pu in the body is primarily based on the 59.5 keV gamma emission of ^{241}Am , the daughter of ^{241}Pu , which is present in reactor-produced Pu. This evaluation is based on the assumption that Am is metabolized in a manner similar to Pu, once inside the living system. This assumption has been shown to be inaccurate (Taylor, 1962; Hammond et al., 1968; Johnson, 1969; Johnson et al., 1970; Watters and Lebel, 1972).

Direct determination of in vivo plutonium which is deposited near the body surface can be achieved by counting the L x-rays of the Pu daughters. These x-rays of 13.6, 17.0 and 20.2 keV (average energy 17 keV), result from the internal conversion of gamma rays from excited states in the uranium daughters. In pure ^{239}Pu there are about four x-rays produced for every 100 alpha particles emitted. In reactor-produced Pu there are isotopes other than ^{239}Pu present and their effect is to increase the ratio of x-rays per alpha particle (Swinth, 1968).

Two major problems arise when counting the low energy uranium L x-rays. One is the problem of rapid attenuation of the x-rays by body tissue; the other relates to self absorption of the x-rays by the Pu particle itself. X-rays with an average energy of 17 keV have a half-value thickness in tissue of about 6 mm and may be detected in most

superficial wounds. Tyler (1966) determined that a spherical particle of Pu with a 0.7 micron diameter will show a measured activity approximately equal to the actual activity. As particle size increases the measured activity is less than the actual activity. For example, a 7 micron diameter particle will show a measured activity about 20 percent less than the actual activity.

Only a few persons are known to have had body burdens in excess of the so-called maximum permissible body burden of $0.04\mu\text{Ci}$ of Pu. According to Evans (1962), individuals with body burdens estimated at $0.4\mu\text{Ci}$ of Pu, or 10 times the maximum permissible body burden, show no symptoms related to internal radiation.

Contaminated Wound Studies

The probable effects of large body burdens of Pu in man are inferred from observations on animals. A large amount of work using laboratory animals for inhalation, ingestion and intravenous injection of Pu has been reviewed by Thompson (1967). Much less work has been done in intradermal, subdermal, or intramuscular implants. One of the earliest studies was that of Lisco et al. (1947). Plutonium was given subcutaneously or intramuscularly to mice, and various manifestations of radiation damage, including malignant fibrosarcomas, were seen. Langham (1962) described the practice of washing and excising wounds from workers as used by Hempelmann in 1944. Seventy-eight wounds were excised during 1944-45 and the excised tissue analyzed for Pu content. Only 3 of the 78 tissues excised by

Hempelmann contained significant amounts of Pu. The practice was discontinued because no method of determining the extent of contamination was available and such practice discouraged workers from reporting minor injuries. Langham (1962) also relates the studies performed at the Los Alamos Scientific Laboratory concerning entrapment and absorption of PuO_2 from contaminated abrasions in the skin of rabbits. Analyses of the carcasses of several animals failed to show absorption of detectable amounts of Pu into the systemic circulation, even though several hundred micrograms of PuO had been applied to the wound. Scott et al. (1948) studied the distribution of Pu following intramuscular injection and found the liver and the skeleton to be major sites of concentration. The effects of intradermal $^{239}\text{Pu}(\text{NO}_3)_4$ injections in blond, miniature swine were studied by Cable et al. (1962). Radioanalysis of selected tissues 7 days after injection showed as much as 12 percent of the administered ^{239}Pu in the regional lymph nodes, about 7 percent in the liver, and about 5 percent in the bone. McClanahan and Ragan (1965) showed that the amount of Pu deposited in soft tissues following subcutaneous injections of $^{239}\text{PuO}_2$ was about 100 times less than that deposited when $^{239}\text{Pu}(\text{NO}_3)_4$ was injected. The regional lymph nodes were an exception, accumulating up to one-half as much Pu when $^{239}\text{PuO}_2$ was injected as when $^{239}\text{Pu}(\text{NO}_3)_4$ was injected. They concluded that the lymphatic system is apparently a major route of translocation for $^{239}\text{PuO}_2$. Taylor (1969) suggested

that lymphatic drainage plays a part in the removal of $^{239}\text{Pu}(\text{NO}_3)_4$ from the intramuscular injection site in rabbits. Studies by Johnson (1969) on lymph transport of air-oxidized $^{239}\text{PuO}_2$, and studies by Bistline *et al.* (1972 a) on lymph transport of $^{239}\text{Pu}(\text{NO}_3)_4$ and high-fired $^{239}\text{PuO}_2$ (850°C) have shown that in dogs the lymphatic system is of great importance in Pu transport from subcutaneous wounds. These studies also showed that the translocation rate of Pu depends on the chemical form of the implanted Pu.

General Characteristics of the Lymphatic System

Although lymphatics presumably had been seen by Herophilos, 300 B.C., and Erasistratus, 310 - 250 B.C., the documented study of lymph dates from 1622, when Asellius demonstrated "lacteals" in the mesentery of a well-fed dog (Mayerson, 1963). The lymphatic system begins peripherally as a network of delicate vessels which drain the tissue spaces. Lymph flows only one way, since lymphatic vessels contain valves which do not permit retrograde lymph flow. The lymphatic system is an important homeostatic mechanism designed (1) to prevent excessive change in interstitial fluid composition and (2) to assist in the maintenance of constant plasma levels of substances that leak from blood capillaries and cannot re-enter them directly (Mayerson, 1963). It is now well-recognized that there is a continuous leakage of proteins and lipids from healthy blood capillaries. All of

the proteins and lipids present in plasma can be demonstrated in lymph (Page et al., 1953; Best and Taylor, 1966).

The distribution of the lymphatic system has been described by Yoffey and Courtice (1970) as follows:

"Although the lymphatic capillaries spread into a tissue after the blood vessels, the density of the lymphatic plexus does not always run parallel with the richness of the blood supply. In the central nervous system there is no invasion by lymphatics, so that in the adult organism there are no lymphatics in this tissue. No one has yet described lymphatic vessels in bone or bone marrow. Recent electron microscopic studies have revealed the fine structure of the marrow sinusoids but no lymphatic vessels were seen. In several other tissues the lymphatic vessels do not penetrate as far as blood vessels: in lobular organs such as the liver and mammary gland the lymphatic capillaries do not penetrate the lobules but are arranged around the periphery; in the lung the lymphatics are not present in the alveolar-blood capillary region; in voluntary muscle the lymphatics are present only in the fascial planes; in spleen and thymus lymphatics have been observed only in the capsule and in the thickest trabeculae."

Interspersed along the lymphatic vessels are lymph nodes. A lymph node is a mass of lymphoid tissues whose function is to filter lymph and to form new lymphocytes. Lymph nodes are small bean-shaped organs, which are whitish in color in the fresh specimen. A description of lymph flow through a lymph node was given by Bevelander (1967) as follows:

"Afferent lymph vessels approach the convex surface of the node and pierce the capsule, opening into the cortical sinuses. From there the lymph passes into the medullary sinuses and is eventually collected at the hilum in the efferent vessels."

A diagram showing the relationship of lymph node to lymphatic vessels is presented in Fig. 1.

Role of Lymphatics in Translocation of Internal Pu Contamination

The role of the lymphatic system in translocation of Pu from lung to lymph node has been studied for many years. In one study (Park et al., 1962) beagles were exposed to $^{239}\text{PuO}_2$ aerosols. Deposition of 0.9-0.1 $\mu\text{Ci/g}$ of lung caused death in 31 dogs in 55-412 days after exposure. Gross and histopathologic tissue changes were limited to the lungs and associated lymph nodes. The bronchial and mediastinal lymph nodes were enlarged two to three times. A study by Bair et al. (1962) compared the tissue distribution of $^{239}\text{PuO}_2$ administered by intravenous injection and by inhalation. In terms of concentration, expressed as nCi of $^{239}\text{Pu/g}$ of tissue, lungs and bronchial lymph nodes were high after inhalation, whereas the liver and spleen were high after IV injection. These studies did not determine the transport mechanism of Pu in lymph, however.

Since the protein composition of lymph and blood plasma are qualitatively the same (Best and Taylor, 1966), a knowledge of Pu transport in plasma may suggest Pu transport mechanisms in lymph. It has been shown that transferrin, an iron-binding β -globulin (MW $\approx$ 80,000) is a major transport protein in the plasma of the rat (Boocock and Popplewell, 1965; Turner and Taylor, 1968), the dog (Stevens et al., 1968) and man (Popplewell and Boocock, 1967; Stover et al.,

Figure 1. Diagram showing relationship of lymph node to lymphatic vessels. (From Bevelander, 1967)

1968). A large fraction of monomeric Pu(IV) introduced into the circulation is bound to transferrin. Turner and Taylor (1968) found 85% in transferrin in rats after 1 hr and Stevens et al. (1968) found 96% in transferrin in dogs after 7 hours. Taylor (1972) states that the study of the reactions of Pu with proteins, either in vivo or in vitro, is complicated by the hydrolytic reactions which make it difficult to distinguish free from protein-bound Pu.

However, as late as 1968, the role of the lymphatic system as an important metabolic pathway from superficial wounds was not generally recognized. A diagram of the principle metabolic pathways of radionuclides in the body as determined by the International Commission on Radiological Protection (ICRP Publication 10) is shown in Fig. 2. The diagram shows no direct pathway from superficial wound to lymph node even though high lymph node concentrations of ^{239}Pu had been shown in animals contaminated via intradermal and subcutaneous implants by Cable et al. (1962) and by McClanahan and Ragan (1965). In 1970 Johnson et al. reported on the kinetics of lymph node buildup of air-oxidized $^{239}\text{PuO}_2$ from a subcutaneous implant. Howells et al. (1972) are presently investigating significant lymph node burdens in a worker who was accidentally contaminated with plutonium oxalate via a puncture wound to the web between the index finger and middle fingers on the palmar surface of his left hand.

Figure 2. Principle metabolic pathways of radionuclides
in the body. (From ICRP 10)

At present an in-depth study of lymph transport of plutonium is being carried out at Colorado State University. These studies range from distribution and excretion studies to studies of the histopathology of the lymph nodes. The chemical forms of plutonium studied to date include air-oxidized $^{239}\text{PuO}_2$, $\text{Pu}(\text{NO}_3)_4$, PuF_4 , and high-fired (850°C) $^{239}\text{PuO}_2$. These studies have been summarized by Lebel et al. (1972).

The need for studies like those presently underway at facilities such as Colorado State University, Battelle Northwest, Lovelace Foundation and Rocky Flats is best expressed in a statement by W. B. Norwood (1972 b):

"Many years ago Langham and associates administered small quantities of plutonium citrate intravenously to men who were suffering from incurable diseases, and determined the daily output of plutonium in urine and feces over a comparatively long period of time. The decay curves relating input with daily output are reasonably accurate for cases in which plutonium citrate is injected in the veins. However, while many accidental depositions have occurred, I do not know of one in which plutonium citrate was injected into a vein. Deposition is usually by inhalation or via flesh wounds, and the plutonium is usually in the form of the metal, the oxide, the nitrate or the fluoride. Most of these compounds are poorly soluble in the body fluids and are metabolized in the body quite differently than is the intravenously-administered soluble citrate."

CHAPTER III

MATERIALS AND METHODS

Dog Assignments

Twenty-six adult beagles and seven mongrel dogs were used in this study. The beagles were obtained from the barrier-maintained colony of the Collaborative Radiological Health Laboratory and the mongrel dogs were obtained from the Surgical-Metabolic Laboratory on the Foothills Campus at Colorado State University. The dogs were assigned to experimental groups as shown in Table 2.

Animal Care

The dogs were maintained in accordance with provisions of the Laboratory Animal Welfare Act (1967). Kennel design is as follows: 15 x 15 feet in size, 1 foot gravel base, cyclone fence (4 ft high, 2 in. mesh), with concrete curbs extending 12 inches above the gravel. Galvanized sheet metal panels on the fence provided shade, and concrete houses with wooden floors provided shelter. The dogs were fed a nutritionally adequate dry ration of dog food. Automatic watering devices provided a constant source of water in the summer, while daily filling of water pans was necessary in the winter. Cleaning and sanitation of feed and water pans was performed on a regular basis.

TABLE 2
Assignment of Dogs to Experimental Groups

Experiment	Group	Number of Dogs	Species	Sex
1st Lymphadenectomy	IA	6 Pu-contaminated 2 Control	Beagle Beagle	M M
2nd Lymphadenectomy	IB	6 Pu-contaminated 2 Control	Beagle	F
Delayed Lymphadenectomy	II	10 Pu-contaminated	Beagle	M
Rapid Pu Translocation	-	2 Pu-contaminated	Mongrel	M
Lymph System Anatomy Studies				
Lymph Vessel Drainage	-	4 Latex-injected	Mongrel	M
Lymph Node Number	-	1 Dye-injected	Mongrel	M

Veterinary medical examinations and treatment of conditions arising in the dogs were provided. All dogs were vaccinated against rabies and distemper.

Implant Procedures

All PuO_2 implants were preceded by preparing the laboratory for handling radioactive materials. Plastic coverings were placed on walls and equipment, while plastic-backed absorbent paper covered the floor. Personnel protective equipment included respirators, coveralls, surgical caps, rubber gloves and booties. These were used during the implanting procedure and as needed following implantation. Health Physics personnel from Rocky Flats surveyed all Pu implants. Air samples and contamination surveys were taken to assure adequate containment during and after implant.

The high-fired $^{239}\text{PuO}_2$ was prepared and provided by Rocky Flats. The physical, chemical and isotopic characteristics of the material are shown in Table 3. The geometric mean count diameter was reported to be 0.7 microns. The plutonium was packaged in rubber diaphragm stoppered vials which contained an average of 1 mg of PuO_2 . The PuO_2 was diluted with 1 cc of sterile, deionized water before implant.

The dogs were given a pre-anesthetic (25 mg propiopromazine hydrochloride) intramuscularly 1 hr before the anesthetic (pentobarbital sodium) was injected intravenously to effect. No side effects to the drugs were seen.

TABLE 3
Physical, Chemical and Isotopic Characteristics of PuO_2
Supplied by Rocky Flats

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

Particle Size

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

Mass Median Diameter approximates 0.7 μ .

All Pu implants were made in the subcutaneous tissue on the dorsal surface of a dog's paw through a 22-gauge hypodermic needle according to the method described by Johnson (1969). Proper positioning of the needle was determined by palpation, ascertaining that no back-flow of blood had occurred. This latter observation indicated that accidental intravenous injection was not made. With a tuberculin syringe and needle, an aliquot of the PuO_2 suspension (0.25 cc) was withdrawn from the stoppered vial. The syringe was attached to the needle previously positioned in the dog's paw, and the implant was made. Upon withdrawal of the needle, an alpha survey meter was used to determine the extent, if any, of surface contamination. The dog's leg was cleaned with hypochlorite solution at least once. When contamination occurred the procedure was repeated until no activity was detected. The dog was placed in a metabolism cage after implant. Estimates of the amount of Pu implanted were made according to the technique reported by Bistline et al. (1972 b).

Lymphadenectomy Procedures

a) Early Lymphadenectomy (Groups IA and IB)

Lymphadenectomy was performed on two groups of dogs at 1, 2, 4 and 8 hrs after Pu implant. The time schedule for lymphadenectomy was based on the data of Johnson et al. (1970) and Bistline et al. (1972 a), which showed that a high percentage of Pu accumulated in the LSCLN in the first 24 hrs after implant. Group IA consisted of

eight male beagles and Group IB consisted of eight female beagles. In each group two contaminated dogs were left intact and two uncontaminated controls underwent lymphadenectomy.

The superficial cervical lymph nodes are described as commonly consisting of three or more nodes (Miller et al., 1964), but in this study only a single, large node was excised. The other nodes are very small and very difficult to identify. Plutonium build-up with time over the LSCLN region was determined by in vivo counting of the 17 keV x-ray complex with a thin NaI(Tl) detector.

The dogs in Group IA were killed 13 days after implant and the dogs in Group IB were killed 11 days after implant. Samples of liver, spleen, hepatic lymph node, axillary lymph node and right and left superficial cervical lymph nodes were taken at necropsy. Activity was determined by placing the NaI detector on the surface of the tissue and counting the 17 keV x-rays. A more accurate determination of the ^{239}Pu activity in the tissues was made by chemical extraction and liquid scintillation α -counting using the method of Keough and Powers (1970). The total tissue weight was used for all tissues except for liver samples. About 10 g of liver tissue were analyzed.

b) Delayed Lymphadenectomy (Group II)

The results obtained in the early lymphadenectomy studies concerning translocation of Pu beyond the LSCLN combined with data

concerning the long term Pu accumulation in the LSCLN (Bistline et al. 1972 a), suggested the design of the delayed lymphadenectomy study.

Ten male beagles ranging in age from 3 1/2 to 5 years were used in this study. Each of the ten dogs was injected with 0.25 cc of a high-fired PuO_2 suspension. Implant procedures used were the same as those described for the early lymphadenectomy studies.

The activity in the left paw and in the LSCLN was determined by counting the 17 keV x-ray complex with a thin NaI detector. The dogs were monitored for 14 days after implant. During this time in vivo accumulation of Pu in the LSCLN was recorded with the NaI detector. After equilibrium was reached on the 14th day after implant the LSCLNs were surgically excised from 5 of the 10 dogs and the activity in the region of the excised node was determined. Daily counts over the implant site in the left paw and in the LSCLN region were made for four successive days after lymphadenectomy. Thereafter, the dogs were counted twice a week. Dogs in Group II were killed 35 days after surgery and were necropsied. The tissues sampled at necropsy were the same as those sampled in the early experiments. Tissue samples were analyzed for ^{239}Pu according to the method of Keough and Powers (1970), to determine whether or not the Pu burdens in the tissues were higher in the lymphadenectomized dogs than in the intact dogs.

Study of Rapid Lymph Transport of Pu

Two methods were investigated for the collection of lymph from a dog's limb. In both methods the afferent lymphatic vessels to the popliteal lymph node were isolated in the femoral region of a dog's hind limb along the dorsal and plantar branches of the lateral saphenous vein. One vessel was chosen for lymph collection and the others were ligated, so that the unrestricted vessel might carry a greater volume of lymph.

One method consisted of placing a plastic trough under the isolated lymphatic vessel and allowing the lymph to drain into a collecting vial. This procedure was used for one dog. The more suitable method (used in the second dog) consisted of cannulating a lymphatic vessel with medical grade polyvinyl tubing using the method of Nelson and Swan (1969). For the implant and collecting procedure the dogs were anesthetized. The lymph vessels were surgically exposed, isolated and cannulated in one dog. In both dogs, lymph flow was stimulated by gently massaging the dog's paw and by intravenously infusing a saline solution. When adequate lymph flow was obtained, a 22 gauge hyperdermic needle was positioned subcutaneously in the dorsal metatarsus for PuO_2 implant. A 0.1 cc implant of the PuO_2 suspension was made and lymph was collected for 2 hours. Collection intervals for the cannulated lymph vessel are listed in Table 4.

TABLE 4
Lymph Sample Collection Intervals

Tube Number	Collection Interval
1	0-15 min
2	15-30 min
3	30-60 min
4	60-120 min

Thirty minutes after implant 4 microscope slides of lymph were prepared for autoradiography by placing 3 drops of lymph on each slide. The slides were stained with Wright's stain before being autoradiographed with Kodak AR-10 stripping film. The slides were developed after 3, 5, 7 and 14 days exposure.

Lymph samples were centrifuged to separate cells from the cell-free fraction. The packed cells were formalin-fixed, paraffin-embedded, and hematoxylin-and eosin-stained before being autoradiographed. Cellular and acellular fractions were prepared for liquid scintillation α -counting using the method of Keough and Powers (1970). Acellular fractions were prepared for disc electrophoresis.

Discontinuous (Disc) Electrophoresis

Disc electrophoresis is a special form of polyacrylamide gel electrophoresis, in which the sample passes through two gels of different pore size and pH. In the first gel, ions migrate into a region of discontinuous pH's and become tightly concentrated into a stack of

contiguous bands. As the migration advances across the boundary into the smaller-pore separating gel, different pH conditions are encountered. Salts pass through the protein layers, and separation in a constant current gradient then proceeds under the combined influence of electrophoretic mobility and molecular sieving by the gel. The double action of first concentrating and stacking, then separating is what gives disc electrophoresis increased sharpness of resolution over single element polyacrylamide or starch systems (Canalco, 1965).

The gel system used covers proteins ranging from 10,000-1,000,000 molecular weight, with maximum resolution from 30,000-300,000 molecular weight. The chemical formulation of the gel system is shown in Table 5, and the electrophoresis apparatus is shown in Fig. 3.

Preliminary tests showed that 150 λ of lymph could be easily separated by the gel and contained sufficient ^{239}Pu to be counted in a liquid scintillation counter. This volume was used for the final analyses.

After the gels were prepared and placed in the electrophoresis apparatus, 200 λ (150 λ lymph + 50 λ of 50% glucose) were layered on the surface of the stacking gel. A current of 0.5 ma per column was applied to the gel until the proteins reached the separating gel. The current was then increased to 1.5 ma per column. Protein separation was completed in ~6 hours.

Figure 3. Apparatus Used in Discontinuous Electrophoresis Procedure. Protein bands can be seen in the stacking gel.

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TABLE 5
Chemical Formulation of Polyacrylamide Gels
(Bamburg et al., 1973)

<u>Separating Gel (7%, pH 9.5)</u>	
1 vol	12 ml in HCl; 9 g. Tris; 0.06 ml TEMED (dilute to 25 ml with H ₂ O)
2 vol	7 g. acrylamide; 0.134 g. Bis (dilute to 25 ml with H ₂ O)
1 vol	H ₂ O
4 vol	0.14% ammo persulphate
<u>Stacking Gel</u>	
1 vol	12.8 ml 1 M H ₃ PO ₄ ; 2.85 g. Tris; 0.10 ml TEMED (dilute to 100 ml with H ₂ O)
2 vol	5.0 g. acrylamide, 1.25 g. Bis (dilute to 100 ml with H ₂ O)
1 vol	0.002 g. Riboflavin (dilute to 100 ml with H ₂ O)
<u>Upper Buffer</u>	
Tris	6.42 g.
Glycine	3.98 g.
H ₂ O	to 1 l.
<u>Lower Buffer</u>	
Tris	12.10 g.
Dissolved in and made up to 1 liter with 0.05 M HCl	
<u>Sample Buffer</u>	
Use Upper Buffer (1:5)	

The gel columns were then removed from the glass tubes, were stained with Coomassie Blue dye, and sectioned to isolate the following protein groups: a) Albumin and α -globulins, b) β -globulins, including transferrin, c) γ -globulins. These groups were identified by comparison with published electrophoretic patterns of serum proteins (Maurer, 1971). The gel sections were prepared for liquid scintillation α -counting using the method of Keough and Powers (1970).

Autoradiography of Lymph Node Sections

Autoradiographs were obtained from lymph node sections of dogs in Groups I and II using Kodak AR-10 stripping film. The lymph nodes had been excised at the following times after Pu implant: 1 hr, 8 hrs, 2 wks and 1 yr. The film was exposed to the α -radiation for 72 hours. The autoradiographic slides were developed, hematoxlin- and eosin-stained, and viewed through a light microscope at a magnification of 160 X. Photomicrographs were made of the sections, and the size of the α -stars seen in each preparation was compared with those seen in the other lymph node preparations. In addition, the size of α -stars in the lymph node sections was compared with the α -stars seen in the packed cells of lymph, which had been prepared for autoradiography in the same way as the lymph node sections and were developed after 72 hrs exposure.

Contrast Studies of Lymphatic Vessels

The anatomy of the lymphatic system in the dog's forelimbs and hindlimbs was studied because observations made in previous experiments at this laboratory have shown that individual dogs may have elevated Pu burdens in the axillary lymph node while other dogs contain a very small burden in this node (Johnson, 1969). No explanation of this difference in Pu translocation was made in the past.

Subcutaneous injections of India ink were made in the dog's forepaws and hindpaws in the dorsal metacarpal and metatarsal areas. The dogs were killed with an overdose of pentobarbital sodium about 10 min after the ink was injected. An incision was made through the skin of the dog's leg, and lymphatic vessels were easily followed, since they were filled with ink. Colored latex was then injected directly into the lymphatic vessels. The skin was reflected and gross observations were made while tracing the latex-filled vessels to a lymph node.

To ascertain the number of lymph nodes in the LSCLN chain of a beagle, 10 cc of a 5% solution of Niagara Sky Blue 6B (J. T. Baker Chemical Company, Phillipsburg, New Jersey), a relatively non-toxic vital dye, was injected intravenously. The 10 kg dog was killed 14 days after injection. During that time the dye was absorbed and then largely removed from the reticulo-endothelial (RE) system (Sanders and Florey, 1940). At necropsy selected lymph nodes were excised and weighed.

CHAPTER IV

RESULTS AND DISCUSSION

The present experimental work was conducted to identify the mechanism by which high-fired $^{239}\text{PuO}_2$ is rapidly translocated from a subcutaneous wound via the lymphatic system, and to determine the effect of regional lymph node excision on subsequent Pu translocation. The first area of research was chosen because investigators at Colorado State University have observed, but have not explained, rapid Pu translocation from subcutaneous wounds to regional lymph nodes. The second area was chosen in order to evaluate lymphadenectomy as a possible therapeutic procedure in Pu wound cases.

The principal experimental model used for these studies was the beagle. The criteria which make the beagle a useful model for systemic studies which may be applied to man have been reviewed by Andersen (1970) and Bustad et al. (1972). Mongrel dogs were used when beagles were not available.

The data generated in this study were obtained by measurement of the 17 keV uranium L X-rays by in vivo counting, by liquid scintillation α -counting, by autoradiography of tissue samples and by disc electrophoresis assays of lymph.

Early Lymphadenectomy Experiments

Accumulation of Pu in the LSCLN of the lymphadenectomized dogs indicated that the residual lymph nodes assumed the filtering function of the excised node within 2 days after excision. The build-up curves from the two early lymphadenectomy experiments (Groups I and II) are presented in Figures 4 and 5, respectively. The low, erratic activity detected in the LSCLN region of the lymphadenectomized dogs for a few days after surgery was due to the fact that the residual lymph nodes were not yet functioning efficiently, and lymph was translocated beyond the LSCLN region. The slopes of the build-up curves for lymphadenectomized dogs parallels the slopes of the curves for the intact dogs 4 to 6 days after surgery. At necropsy LSCLN samples with detectable Pu were obtained from all lymphadenectomized dogs. Between the time of excision of the primary node and time of necropsy (11 to 13 days), the residual lymph nodes had enlarged to the size of the original lymph node, Table 7.

The liver, spleen and hepatic lymph nodes in the lymphadenectomized dogs accumulated greater burdens of Pu than did the same organs in the intact dogs. The data from the early experiments are shown in Figure 6. These data suggest that much of the Pu translocation beyond the LSCLN occurred before the residual lymph nodes began to function efficiently. The rate at which the residual lymph nodes begin to accumulate Pu may be a major factor in determining

Figure 4. Change in the Fraction of Pu Accumulation in the Left Superficial Cervical Lymph Node with Time. Before and After Lymphadenectomy (Group IA)

Figure 5. Change in the Fraction of Pu Accumulation
in the Left Superficial Cervical Lymph Node
with Time. Before and After Lymphadenectomy
(Group IB)

Figure 6. Fraction of ^{239}Pu in Selected Tissues ($\bar{X} \pm \text{SE}$). Data are from Early and Delayed Lymphadenectomy Experiments.

L = Liver

S = Spleen

HLN = Hepatic Lymph Node

how much Pu translocates to other organs. The greater and more rapid lymph node build-up of Pu in Group IB might be explained by improved surgical techniques which caused less trauma to tissues in the LSCLN region. Since Group IB consisted entirely of female beagles and Group IA consisted entirely of male beagles, the question of sex differences in Pu build-up arose. However, the male dogs in the delayed lymphadenectomy study, Group II, also showed rapid Pu build-up in the LSCLN region after surgery. These results tend to support the idea that improved surgical techniques and not sex differences are the reason for the more rapid Pu build-up in the LSCLN region after surgery.

The early experiments suggested that there may be an optimal time range (10-20 days after implant) during which the LSCLN might be excised, to remove a maximal amount of Pu, while permitting a minimal amount of Pu to translocate beyond the lymph node. The results from the early lymphadenectomy experiments combined with data concerning the long-term accumulation of Pu in the LSCLN (Bistline et al., 1972 a) suggested the design of the delayed lymphadenectomy experiment.

Delayed Lymphadenectomy Experiment

The build-up of Pu in the LSCLN region was determined by taking daily counts with a NaI(Tl) detector before and after lymphadenectomy. These data are shown in Figure 7. The rapid translocation

Figure 7. Change in the Fraction of Pu Accumulation in the Left Superficial Cervical Lymph Node with Time. Before and After Lymphadenectomy ($\bar{X} \pm SE$) (Group II)

■ = Dog #1440

● = Dogs with LSCLN Intact

▲ = Lymphadenectomized Dogs

of a large amount of Pu in dog #1440 could have been due to accidental implantation of the PuO_2 either directly into a lymphatic vessel or in very close proximity to a lymphatic vessel. The built-up data from dog #1440 were included with the lymphadenectomized dogs immediately after surgery, since build-up in the residual lymph nodes paralleled that seen in other lymphadenectomized dogs. The build-up of Pu in the LSCLN seen in this experiment is consistent with that seen in the long-term, high-fired PuO_2 experiments conducted by Bistline et al., (1972). No equation was determined for the build-up curves seen in Figure 7, since Bistline et al. (1972 a) show that after day 50 the Pu build-up curves level off and then exhibit a negative slope. The curves in the delayed lymphadenectomy experiment were determined only to day 49. They leveled off by day 10 but did not develop a negative slope. The dogs were killed 35 days after surgery and were necropsied. The tissues sampled underwent radiochemical analysis for ^{239}Pu by liquid scintillation α -counting. The overall recovery of the procedure was determined to be 90% using tissues spiked with known amounts of $^{239}\text{PuO}_2$.

It was hypothesized that the leveling-off of the Pu build-up in the LSCLN was due to a decreased translocation rate of Pu from the implant site to the LSCLN. The build-up curves (Figure 7) show that this is the case. The residual LSCLN accumulated Pu 10 to 100 times more slowly than did the original LSCLN. The leveling-off of

the LSCLN build-up may also be due to Pu translocating beyond the LSCLN. Translocation from the LSCLN in intact dogs has been demonstrated by Johnson (1969) and Bistline et al., (1972 b).

The relationship between tissue burdens in intact versus lymphadenectomized dogs parallels that seen in Groups IA and IB (Figure 6). It was hypothesized that delayed lymphadenectomy would allow for maximal Pu removal with the excised node, while allowing minimal Pu translocation beyond the LSCLN chain.

According to Thompson (1967) the spleen, under certain conditions, may accumulate Pu concentrations as high or higher than those in liver or bone. Conditions that favor deposition in liver seem also to favor deposition in spleen. However, spleen Pu concentrations are usually lower than those in liver if retention in the liver is high as in man, dog and pig. The data in Figure 6 parallel the Pu deposition described by Thompson (1967). The hepatic lymph node also shows significant Pu accumulation.

The therapeutic value of lymphadenectomy for management of Pu-contaminated wounds in humans may be improved by considering it in conjunction with chelation therapy and/or excision of the contaminated wound site which should minimize the Pu reservoir.

Since the concept of "dose commitment" (rem to critical organ of reference man per μCi) rather than "organ burden" is expected to become the basic standard for internal dose (Morgan, 1972), it

✓

becomes important to consider the total dose an individual will receive during his lifetime. Morgan (1972) points out the importance of removing as much ^{239}Pu as possible from the body of a contaminated worker. He provides the example of a worker accumulating a bone burden equal to one-half of the maximum permissible organ burden, $q/2$, (sic) which would correspond to an average dose rate to the skeleton of 30 rem/yr. This would lead to an excessive skeletal dose during the following 40 years and would restrict all his future employment as a radiation worker. Morgan (1972) also states that a bone burden of $q/2$ would yield a 7% chance that the worker may die of radiation-induced cancer.

Since translocation of $^{239}\text{PuO}_2$ beyond intact lymph nodes to more radiosensitive tissues has been shown to occur (Johnson, 1969; Bistline *et al.*, 1972 a, b), a long-term build-up and concentration of ^{239}Pu in the skeleton and liver may occur from Pu initially concentrated in lymph nodes. The total "dose commitment" to these organs would then be in excess of that allowed by the recommendations of the ICRP (1966). Lymphadenectomy, chelation therapy and wound excision should decrease the individual's "dose commitment."

Rapid Lymph Transport of Pu

Lymph was collected from the hind limb of two dogs. In the first dog the lymph was collected in two clear fractions before excessive contamination with blood occurred. In the second dog lymph was

collected in four fractions. All samples were allowed to clot before being centrifuged to separate the cellular and acellular fractions. No Pu contamination was detected with a portable α -survey meter during any of the procedures.

After centrifugation the cellular and acellular fractions of lymph from the tubes were separated. Both fractions were prepared for α -counting. The ratio of ^{239}Pu in the cellular fraction versus the acellular fraction in both dogs was approximately 2:1 (Table 6).

TABLE 6
Ratio of ^{239}Pu in Cellular vs Acellular Lymph Fractions
(Mean $\pm$ S. D.)

Cellular Fraction	Acellular Fraction
1.98 $\pm$ 0.193	1.00 $\pm$ 0.048

This result suggests that a major process of PuO_2 translocation in lymph is by phagocytosis of PuO_2 particles which enter the lymphatic system. Sanders (1972) and Sanders and Jackson (1972) have described phagocytosis of PuO_2 particles by macrophages. The acellular lymph fraction, which contained about one-third of the rapidly translocated Pu, was analyzed by disc electrophoresis to determine the transport mechanism of PuO_2 in lymph.

Disc Electrophoresis Results

Since Pu in blood plasma has been shown to be protein-bound (Boocock and Popplewell, 1965; Popplewell and Boocock, 1967; Stevens et al., 1968; Stover et al., 1968; Turner and Taylor, 1968), the Pu present in the acellular lymph fraction, which is qualitatively identical with plasma, was assumed to be protein-bound. Therefore, disc electrophoresis, a technique capable of separating proteins, was used to determine whether or not the Pu carried in lymph was protein-bound.

Five replicate lymph samples from each of the two dogs were run in order to assess the reproducibility of the procedure. All 10 gels contained the same protein bands. A sketch of the protein separation in the gels is presented in Figure 8. Three protein bands are indicated along with the grouped globulin fractions.

Karlson (1968) reported that at least 15 major protein groups of blood plasma have been identified. Only 11 protein bands were seen in the lymph samples analyzed. The fact that lymph contains only 50% as much protein as plasma (Best and Taylor, 1966) may explain the smaller number of protein bands seen in the lymph samples analyzed. Maurer (1971) shows that the proteins seen in polyacrylamide gels are dependent on the origin of the sample.

After the gels were removed from the glass tubes they were sectioned for liquid scintillation α -counting. The results of the α -counting are shown in Figure 9. These results show that the greater

Figure 8. Sketch of Polyacrylamide Gel Showing Individual Protein Bands. The gel was sectioned into three globulin fractions for liquid scintillation α -counting.

Figure 9. Percentage of Protein Bound ^{239}Pu in the Protein Fractions of Dog Lymph.

percentage of Pu is associated with the β -globulin fraction (including transferrin). These results, which are similar to those seen for Pu binding by plasma proteins (Boocock and Popplewell, 1965; Popplewell and Boocock, 1967; Stevens et al., 1968; Stover et al., 1968; Turner and Taylor, 1968), were not unexpected, since the protein composition of lymph is qualitatively identical to the protein composition of plasma. Therefore, the binding properties of proteins, such as transferrin, should be similar in lymph or plasma.

Autoradiography Results

The autoradiographs made on drops of whole lymph applied directly to a microscope slide yielded no detectable α -tracks. The preparation of the slides for staining and autoradiography may have removed the Pu, whether it was free, protein-bound or cellularly trapped.

The autoradiographs of the cellular fraction of lymph, developed after 72 hours exposure, showed a wide range of $^{239}\text{PuO}_2$ particle sizes. The size range in the packed cells was estimated to be 0.1 to 1.0 μ . This is illustrated in Figure 10. Whether the larger particles were single particles or agglomerations of small particles was not determined. Particle agglomeration in the CSU Pu-wound project is being studied by Dagle (1973) using X-ray diffraction techniques. Particle sizes were estimated optically from the autoradiographs using

Figure 10. Typical Size Range of α -Stars of $^{239}\text{PuO}_2$ in
Packed Cells of Lymph Collected 1 hr.
Post-Implant. 72 hr. Exposure, (1066 X
magnification)

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the methods of Leary (1951) and Moss et al. (1964). These results indicate that the phagocytic cells in lymph are capable of transporting $^{239}\text{PuO}_2$ particles of varying size.

The autoradiographic study of lymph nodes excised at different times after implant (1 hr, 8 hrs, 2 wks, and 1 yr) was used to determine whether there was more rapid transport of large or small $^{239}\text{PuO}_2$ particles via lymph.

The results of this study are shown in Figure 11. The α -stars seen are typical of all fields examined on the slides. The 1 hr and 8 hr lymph node sections contained only small α -stars, while the 2 wk and 1 yr sections contained a wide range of α -star sizes. The number of α -stars per field was low on the 1 hr and 8 hr specimens, and high on the 2 wk and 1 yr specimens.

These results suggest that the $^{239}\text{PuO}_2$ which is most rapidly translocated to the LSCLN is protein-bound. If the cellular fraction of lymph were to move as rapidly as the acellular fraction, larger $^{239}\text{PuO}_2$ particles would undoubtedly be found in lymph node sections excised shortly after Pu implant. No large α -stars were seen in the lymph node sections excised on the day of Pu implant, but a very wide size range of α -stars was seen in all lymph node sections excised after two weeks.

The conclusion made from these results is that lymphatic translocation of $^{239}\text{PuO}_2$ is divided into at least two phases. During the

Figure 11. Change in Particle Size of $^{239}\text{PuO}_2$ Particles
in the LSCLN of Beagles with Pu Accumulation
Time.

- a. 1 hr $^{239}\text{PuO}_2$ accumulation
- b. 8 hr $^{239}\text{PuO}_2$ accumulation
- c. 2 wk $^{239}\text{PuO}_2$ accumulation
- d. 1 yr $^{239}\text{PuO}_2$ accumulation

All samples autoradiographed for 72 hrs.
500 X magnification

first phase small protein-bound $^{239}\text{PuO}_2$ is transported to the regional lymph node within minutes. Enough $^{239}\text{PuO}_2$ is translocated via this mode that the low energy U L x-rays can be detected, in vivo. The larger $^{239}\text{PuO}_2$ particles may be phagocytized by cells in the lymph and may be transported to the lymph node more slowly. By 2 wks, however, the size range of α -stars is similar to that seen at 1 yr.

The lymphatic system obviously has the capacity to rapidly carry foreign matter from the site of entry to a lymph node, where it can be retained. The rapid translocation of Pu via the lymph is surprising when one considers that all implants were made while the dogs were under pentobarbital sodium anesthesia. This anesthetic has been shown to markedly reduce lymph flow (Polderman et al., 1943; Hungerford and Reinhardt, 1950).

Lymphatic System Anatomy

The India ink and latex injections in the dog's left forepaw outlined a lymph vessel which loops around the distal portion of the first metacarpus. Afferent vessels to this looping vessel come from the other metacarpals. The gross anatomical study attempted yielded the following results. The portion of the looping vessel which is dorsal to the first metacarpal bone was followed to the LSCLN, while the portion of the lymph vessel which is ventral to the first metacarpal bone was followed to the left axillary lymph node.

The elevated axillary lymph node burdens may, therefore, be explained by these findings. Any Pu which is picked up by the ventral lymph vessels may go to the axillary lymph node instead of the LSCLN.

The gross studies of the anatomy of the hind paw outlined lymph vessels which all went to the popliteal lymph node. Since only one pathway is seen in the hind leg, it may be the preferable site for Pu implant in future studies of lymph transport in beagles.

Reports indicate that the number and size of lymph nodes in the superficial cervical lymph node chain in dogs may vary (Miller et al., 1964; Chretien et al., 1967; Ratzlaff, 1970). The dog used to ascertain the size and number of LSCLN's was killed 14 days after injection of the Niagara Sky Blue dye. All lymph nodes were stained dark blue, while all surrounding tissues were stained light blue. The lymph nodes could be easily seen.

The right superficial cervical lymph node chain consisted of three lymph nodes, and the LSCLN chain consisted of two lymph nodes. These findings are in agreement with descriptions in the literature concerning the number of lymph nodes in the superficial cervical lymph node chain.

The weights of the lymph nodes sampled are shown in Table 7. These are consistent with those seen in all lymphadenectomy experiments and in long-term experiments conducted at this laboratory.

These results explain why only one LSCLN was seen in the lymphadenectomy experiments. The smaller nodes in the chain were not distinguishable from the surrounding tissues. Therefore, only a single, large node was excised.

TABLE 7.
Lymph Node Weights (grams) $\pm$ SD

Group	Excised LSCLN	LSCLN at Necropsy
Group IA	0.453 ± 0.138	1.020 ± 0.305
Group IB	1.091 ± 0.027	1.057 ± 0.494
Group II	1.135 ± 0.213	1.508 ± 0.445
Three-month PuO ₂ Experiment	--	1.637 ± 0.780
Dye Study		
Ventral		
Right	--	0.783
Left	--	1.105
Dorsal		
Right	--	0.145, 0.068
Left	--	0.117

CHAPTER V

SUMMARY

The major goals of this study were twofold: a) to study the effect of lymphadenectomy in PuO_2 translocation, and b) to gain new information concerning the mechanisms of rapid lymph transport of PuO_2 to regional lymph nodes. Minor anatomical studies supplied answers to questions which arose during the course of the study.

The potential for long-term bone and liver accumulation of Pu, and the serious consequences of such accumulation, make it imperative to remove as much Pu from the body as possible as soon as possible. The lymphadenectomy studies suggest that lymph node excision may be an effective therapeutic procedure in Pu wound management. Chelation therapy and/or wound site excision may increase the therapeutic value of excision of Pu-contaminated lymph nodes.

The transport of PuO_2 in lymph appears to be primarily by way of the cellular fraction. The range of particle sizes seen in the cellular fraction was estimated to be from 0.1 to 1.0 μ . The fraction of PuO_2 transported by the acellular lymph fraction was shown by disc electrophoresis to be primarily associated with the β -globulin fraction, which includes transferrin, the major Pu-transport protein in blood plasma.

Autoradiographic studies on sections of lymph nodes, which were excised at 1 hr, 8 hr, 2 wk and 1 yr, showed that the translocation of very small PuO_2 "particles" from the wound site to the lymph node occurred during the first day after PuO_2 implant. By 2 wk large PuO_2 particles could be found in the lymph node. The range of particle sizes seen at 2 wk was the same as that seen in the lymph node sections taken 1 yr after implant.

Since large PuO_2 particles were present in the lymph 1 hr after implant, but no large PuO_2 particles were seen in the lymph node sections excised 1 hr after implant, it is felt that the rapid Pu translocation to the lymph node occurs via the proteins of lymph, rather than by phagocytosis of the PuO_2 particles. The PuO_2 which is phagocytized probably reaches the lymph node much later than the protein-bound Pu.

The minor anatomical studies showed that the high axillary lymph node burdens of Pu seen in earlier experiments were due to implants being made near the medial portion of the dog's paw. The PuO_2 was then translocated to the axillary lymph node rather than to the superficial cervical lymph node.

The lymphatic system has been shown to be a significant route by which PuO_2 can be translocated from simulated puncture wounds. Further studies are needed to assess the long-term effect of Pu in lymph nodes.

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