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THE UNIVERSITY OF ALBERTA

^{113m}In-INDIUM OXINATE:
A NEW POSSIBLE LUNG SCANNING AGENT

by

© John R. Scott

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ^{113m}In-INDIUM OXINATE: A NEW POSSIBLE LUNG SCANNING AGENT submitted by John R. Scott in partial fulfilment of the requirements for the degree of Master of Science in Radiopharmacy.

Supervisor

Co-Supervisor

Date Dec. 28, 1972

ABSTRACT

The potential use of ^{113m}In-indium oxinate in nuclear medicine was investigated.

Upon intravenous injection of an ethanolic solution of ^{113m}In-indium oxinate, the majority of the radioactivity was found to remain in the circulation for a considerable length of time.

A method of preparation of ^{113m}In-indium oxinate particles having a particle size between 10 and 30 microns was developed. When injected intravenously, the bulk of these particles were trapped in the capillaries of the lungs. Long-term distribution studies in mice of indium oxinate particles labeled with indium-114m indicated that the radioactivity had a half-time in the lungs of about 26.1 hours. Excretion from the body was relatively slow and the excretion curve was found to be composed of three exponential components, with half-times of 1.3 $\pm$ 0.1 days, 4.1 $\pm$ 0.1 days and 18.3 $\pm$ 0.3 days, and a large bound or very slowly excreted portion. The indium did not appear to be excreted as the oxinate.

The chemical toxicity of indium oxinate and oxine appeared to be relatively low. However, after an intravenous dose of 100 mg of indium oxinate suspension per kg body weight into mice, some pathological changes were seen in the lungs.

Lung scans of a rabbit and a dog were obtained after the intravenous administration of ^{113m}In-indium oxinate suspension. These scans indicated that a large proportion of the radioactivity was cleared from the circulation by the capillaries of the lungs.

The radiation dose from 1 mCi of ^{113m}In-indium oxinate was estimated to be 0.02 rad to the whole body, 0.06 rad to the liver and 0.67 rad to the lungs.

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To Shirley

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INTRODUCTION

The use of radioisotopes in organ visualization has increased greatly since parent-daughter generator systems have become commercially available. Of the currently available short-lived isotope generator systems, the ^{99}Mo-^{99m}Tc generators are the most widely used. However, since ^{99}Mo has a relatively short half-life of 67 hours (1), these generators have a useful life of only about one week, which makes their use commercially unfeasible in smaller institutions and in many parts of the world.

The parent-daughter combination of ^{113}Sn-^{113m}In was suggested as a generator system by Greene et al. (2) and by Stang and Richards (3). The system, developed by Subramanian and McAfee, consisted of a zirconium oxide column loaded with ^{113}Sn. The daughter ^{113m}In was eluted with 0.05N HCl (4).

Tin-113 has a half-life of 118 days (1) and therefore such a generator has a useful life of several months. Indium-113m has a half-life of 100 minutes and decays by monoenergetic gamma emission of 393 KeV; approximately one-third of the emissions result in conversion electrons (5).

Radiopharmaceuticals labeled with ^{113m}In have been used for scanning of the lung (6), liver (7), blood pool and brain (8,9) and kidneys (10).

When using a short-lived radioisotope such as ^{113m}In for the preparation of a radiopharmaceutical it is important that the procedure be rapid and uncomplicated. For a cation

such as indium, one method of chemical preparation that meets these criteria is chelation. Chelates of ^{113m}In with ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) have been used in nuclear medicine (9,11,12,13). With these chelates, however, the only significant localization observed is in the kidney and at the site of brain lesions.

Oxine (8-hydroxyquinoline, 8-quinolinol) has been used to a considerable extent in analytical chemistry for the identification and quantitation of a number of cations including indium (14). The chelation of indium by oxine is quantitative (15).

Chelates of EDTA and DTPA are hydrophilic while chelates of oxine are hydrophobic. This study was undertaken to determine if this latter property would result in a specific tissue uptake of ^{113m}In-indium oxinate. Preliminary investigation indicated that intravenous administration of indium oxinate did not show any favorable tissue to blood ratio. However, it was found that precipitation of indium oxinate from solution resulted in particles with a size range optimal for removal by the capillaries of the lung from the circulatory system after intravenous injection. This aspect was further investigated in order to evaluate ^{113m}In-indium oxinate as a possible lung scanning agent. Parameters that were studied included tissue localization of the nuclide, toxicity of the chemical form, elimination

of the agent from the body, and radiation dose from the radiopharmaceutical.

SURVEY OF THE LITERATURE

A. Indium

1. Historical

Indium has an atomic number of 49 and an atomic weight of 114.82, and was discovered in 1863 by Reich and Richter (16). There are twenty-four known isotopes of indium with atomic weights ranging from 106 to 124. Only two of these are found naturally, the radioactive indium-115 (half-life 6 X 10^4 years, 95.77% abundance) and the stable indium-113 (4.23% abundance) (1).

Indium is used commercially for the surface protection of metals to prevent corrosion, and is added to dental alloys, glass-to-glass seals, motion picture screens, cathode oscillographs, transistors and infra-red detectors (17). Indium-114m (half-life 50 days) has been used as a radioactive tracer. Since it has become commercially available, indium-113m (half-life 100 minutes) has become relatively widely used in nuclear medicine in organ scanning techniques (18).

2. Absorption, Distribution and Excretion of Indium

The absorption, distribution and excretion of ^{114m}In-labeled indium after intratracheal, subcutaneous, intramuscular and oral administration in rats was investigated by Smith and associates (19). They found that only 0.5% to 2% of the oral dose of indium trichloride was absorbed from the gastrointestinal tract. Four days after administration, 40.6% of the intratracheal, 14.4% of the subcutaneous and

38.7% of the intramuscular dose of indium remained at the site of application. The comparison of the figures, however, may not be valid, as in each case the indium was administered in a different chemical form. In addition, the authors considered that a substantial portion of the loss from the intratracheal site was due to ciliary action.

Smith et al. (19) found the major sites of initial distribution of indium after subcutaneous and intramuscular injection in rats to be the pelt, skeleton, liver, muscle, kidney and spleen, in that order. The kidney had the largest concentration on a per gram basis. The excretion curve of indium after intramuscular and subcutaneous administration was found to have two components. The phase of rapid excretion had a half-time of about three days following subcutaneous administration and 6.5 days after intramuscular administration. The half-times of the slow phase were 94 days and 126 days following subcutaneous and intramuscular injection, respectively. Half of the administered dose was excreted in 44 days by the subcutaneous group and in 80 days by the intramuscular group. Again, these results may have been influenced by the chemical form of indium used, as the subcutaneous injection was in the form of indium citrate while the intramuscular injection consisted of indium trichloride.

Downs et al. (20) also found that, in the rat, the oral absorption of indium trichloride was limited. Their

results indicated that indium sesquioxide was absorbed to an even lesser extent than indium trichloride from the gastrointestinal tract.

Castronovo and Wagner (21) investigated the distribution and excretion of indium after the intravenous administration of ionic indium (chloride) and colloidal hydrated indium oxide in mice, using ^{114m}In as a label. Immediately after injection, most of the ionic indium was found in the blood. As the concentration in the blood fell there was an increase in the concentration in the liver and kidney. The excretion curve was found to have two components. Depending upon the dose used, the fast component had a half-time of 1.9 to 2.1 days and accounted for 31% to 52% of the dose while the slow component had a half-time of 62 to 74.5 days.

As was expected, most of the indium administered in the form of colloidal hydrated oxide was accumulated in the liver and spleen almost immediately after administration (21). At dose levels of 0.2 mg to 0.825 mg of indium there was some translocation of the indium from the liver to the skeleton, kidneys, spleen, and muscle. Excretion of colloidal hydrated indium oxide also had two phases. The fast component had a half-time of 2.0 days and accounted for 18% to 28% of the dose. The slow component had a half-time of 61.9 to 73.8 days, depending on the dose.

3. Toxicity of Indium

The toxicity of indium in various chemical forms has been investigated by a number of workers. Von Oettingen (22) found that the minimum lethal dose of indium (as the citrated chloride) was about 0.06 g per kg when administered subcutaneously in mice. McCord and co-workers (23) found the minimum lethal subcutaneous dose of indium, as the sulfate or citrated chloride, to be 2 mg per kg in rabbits and 10 mg per kg in rats. This same group (24) found that toxicity of orally administered indium sulfate was considerably lower. In the rat, a daily dose of 116 mg per kg produced an ill-defined sub-normal state after 40 days.

Downs et al. (20) studied the toxicity of indium trichloride and indium sesquioxide (In_2O_3). In rats, they found the LD_{50} of indium trichloride (pH 3.4) to be 1.8 mg per kg after intraperitoneal administration and the LD_{50} of intravenously injected indium trichloride in citric acid (pH 7.2 - 7.4) to be 4.1 mg indium per kg. When administered intraperitoneally the principal site of injury was the liver. However, upon intravenous injection the kidney was found to be the only organ showing lesions. Some slight hematological changes were also seen after the intravenous administration.

This group (20) also investigated the toxicity of indium sesquioxide. Single intravenous doses as a 5% suspension produced weight loss in rabbits at doses as low as 30 mg of In_2O_3 per kg. The lungs were the main site of

toxicity with necrotizing pneumonia and edema always present. There was occasional injury to the liver and kidney. Indium sesquioxide administered intravenously at a dose of 30 mg per kg to rats showed only a slight toxicity. Orally administered indium sesquioxide (8% in the diet) in rats produced no decrease in growth and no effects on organ weights or histopathology. Oral indium trichloride (4% in the diet) produced a moderate depression of growth, a mild anemia and slightly increased weight of the lung.

Castronovo and Wagner (21) reported on the toxicity of indium trichloride and colloidal hydrated indium oxide administered intravenously to mice. The colloidal hydrated indium oxide was found to be more toxic, with an LD_{50} of 0.323 mg of indium per kg. The LD_{50} of ionic indium (as the chloride at pH 3.0) was 12.5 mg of indium per kg of body weight. The effect of the higher doses of indium appeared to be mainly on the proximal convoluted tubules of the kidney with some necrosis of the parenchymal cells of the liver. The higher doses of the hydrated indium oxide produced extensive damage to the liver with some damage to the spleen and kidney. There were some hematological changes noted, particularly an acute drop in the platelet count.

Usher et al. (25) investigated the long term effects of a single intravenous dose of indium chloride in rats. At time of sacrifice, six months after administration of 0.67 mg

indium per kg the animals had depressed weight, total protein and alkaline phosphatase levels, but no pathological abnormalities were visible.

In evaluating ^{113m}In-labeled indium hydroxide as a lung scanning agent, Stern and associates (6) found the minimum lethal dose of the indium hydroxide to be about 3 mg per kg in rabbits and 16 mg per kg in mice. Histological examination indicated that the animals died of severe liver damage and necrosis of the kidney tubules.

Ferm and Carpenter (26) reported that indium nitrate was teratogenic in hamsters; doses above 1 mg per kg administered intravenously were completely embryopathic.

B. Oxine

1. Chemistry of Chelation

The chemistry of oxine has been reviewed by several authors (27,28,29). The structure of oxine is shown in Figure 1 (28).

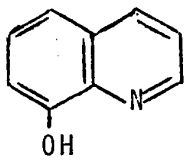


Figure 1

Structure of Oxine

To function as a chelating agent, a molecule must fulfill at least two conditions: a) it must contain at

least two functional groups capable of donating a pair of electrons to the metal atom; b) these functional groups must be so situated in the molecule that the formation of a ring, with a metal atom as the closing member, is possible (30). These functional groups may be basic coordinating groups or acidic groups that have lost a proton. Rings composed of five or six members are the most stable and the most common (30).

Oxine meets these two requirements and can form stable five member ring complexes with at least fifty metals (31). It has been used to a wide extent in analytical chemistry. Precipitation or extraction into organic solvents followed by the spectrophotometric, fluorometric or polarographic analysis of the oxinate has been used for the detection of a number of metals (31).

The application of oxine in the analytical chemistry of indium has been reviewed by Busev (16). The use of oxine to determine indium was first suggested by Geilmann and Wrigge (32). These authors found that indium oxinate has a composition corresponding to the formula $In(C_9H_6NO)_3$ and contains 20.99% indium.

2. Medicinal Uses of Oxine

The antibacterial and antifungal activity of oxine has been investigated to a considerable extent by Albert and associates (33,34,35,36). This work has been reviewed by Albert (37) and by Hollingshead (38). The action of

oxine appears to be mediated by increasing the toxicity of cuprous, ferric or ferrous ions present. Its site of action is probably the cytoplasmic membrane (39). Oxine was found to be without chemotherapeutic effect in mice (36). The compound's antibacterial action was inhibited by red blood cells (34). These may release a thermolabile substance that inhibits the antibacterial action of oxine but not its chelating ability (40).

Oxine has been used in medicine to only a limited extent. Ashurst (41) advocated its use as a 0.5% ointment in the treatment of acne vulgaris and rosacea. The halogenated derivatives of oxine appear to be more potent than oxine itself as intestinal antiseptics. This may be due to the more rapid absorption of oxine from the gastrointestinal tract (42). The most widely used halogenated derivative is iodochlorhydroxyquin which is available in tablet, cream, ointment, powder and suppository form (43).

3. Absorption, Metabolism and Excretion of Oxine

Relatively little work has been done on the metabolism of oxine and its chelates. Grabbe (44) found that in dogs, oxine was rapidly absorbed from the intestine and excreted largely by way of the urine mainly as the sulfate. Heseltine and Freeman (42), using ultraviolet absorption spectrometry, reported that following an oral dose, rats excreted 60-70% of the oxine as a conjugate in the urine within one day. Only small quantities remained in the intestinal contents.

Smith (45) reported that when doses of 250 mg of oxine per kg were administered orally to rabbits, 20% of the dose was recovered in the urine as the glucuronic acid and 6% as the sulfate conjugates.

4. Toxicity of Oxine

Bernstein and associates (46) found that the intraperitoneal LD_{50} of oxine was 48 mg per kg in mice and that of cuprous oxinate was 67 mg per kg. Kadota (47) reports the intravenous lethal dose of oxine in dilute hydrochloric acid to be 50 mg per kg in mice and 65 mg per kg in rabbits.

Kadota also investigated the ability of oxine to produce experimental diabetes mellitus in rabbits. Doses of 10 to 29 mg of oxine per kg, when administered intravenously, caused transitory hyperglycemia. At doses of 50 mg per kg an initial hyperglycemia followed by hypoglycemia and then permanent hyperglycemia occurred (47).

The diabetogenic effect of oxine in rabbits was further investigated by Root and Chen (48). They concluded from this study that the lethal dose of oxine was 65 to 75 mg per kg if a 1% solution was injected intravenously at a rate of 10 ml per minute. At a slower rate of injection the lethal toxicity of oxine was reduced. They also reported that oxine at 50 mg per kg produced hyperglycemia in most of the rabbits used. However, this effect was found not to be permanent as previously stated by Kadota. These same authors

mentioned that the intravenous administration of oxine to rats, hamsters, guinea pigs, cats and dogs did not have a diabetogenic effect (48).

Oxine has been implicated as being a possible carcinogen. Boyland and Watson (49) and Allen et al. (50) found that oxine produced a significant number of tumors when introduced into the bladders of mice in pellets of cholesterol. Hueper (51) reported a slightly higher incidence of tumors in rats when an oxine suspension had been instilled either intravaginally or intrarectally. However, in an extensive study in which rats were fed 0.8% oxine in the diet for up to eighteen months, Yamamoto and associates (52) discovered no evidence of neoplastic change attributable to the treatment. The most significant change that this group observed was the increased deposition of iron in the spleen, liver, kidney, adrenals and thyroid of those animals fed oxine. However, the cellularity of the sternal marrow and the hematocrits of these animals were normal.

C. Use of Radioisotopes in the Evaluation of Pulmonary Function

Radioisotopes have made possible the measurement of regional pulmonary function with a minimum of discomfort and hazard to the patient. The evaluation of pulmonary function is concerned with two basic aspects, ventillation and perfusion. Radioisotopes can be used to measure both of these aspects.

1. Radioactive Gases

The first suggestion for the use of radioisotopes in the diagnosis of lung disease was by Knipping et al. (53). This group used xenon-133 to aid in the early diagnosis of bronchial carcinoma. Xenon-133 has remained the most widely used radioactive gas in the evaluation of pulmonary function.

In the original procedure the patient breathed ^{133}Xe in a closed system and external detectors over various regions of the lung were used to evaluate ventillation. Ball and associates (54) modified this technique by dissolving ^{133}Xe in saline and injecting the solution intravenously. Since the solubility of xenon in an aqueous solution is low, the ^{133}Xe diffuses into the air of the alveoli upon reaching the lung. If the patient holds his breath for several seconds, external scintillation detectors can be used to measure regional pulmonary blood flow. By having the patient rebreath the gas for several minutes before a second determination is made, a measurement of lung size is possible.

Radioisotopes of the three principal respiratory gases: oxygen, carbon dioxide and nitrogen, have been used to a limited extent for lung function studies. ^{15}O-Oxygen (55), ^{15}O-carbon monoxide (56), ^{15}O- or ^{11}C-carbon dioxide (57,58) and ^{13}N-nitrogen (59) have been investigated. Although these isotopes may have advantages over ^{133}Xe, they all have a half-life in the order of minutes and therefore

close proximity of a cyclotron is required. For this reason they have not become widely used.

2. Labeled Particles

If particles of the correct size are injected intravenously they will be trapped in the first capillary bed encountered, that of the lung. Weibel (60) calculated the number of human lung capillary segments to be 2.8×10^{11} with a mean capillary diameter of 8.2 microns. It has been found that labeled particles 10 to 50 microns in size can be used to determine the regional pulmonary blood flow. Wagner and associates (61) pointed out that this method is based on six assumptions:

i) the particles are uniformly mixed in the blood before reaching the pulmonary artery,

ii) hemodynamic and gravitational forces affect the distribution of particles in a similar manner to that of red blood cells,

iii) the particles are nearly completely extracted in a single passage through the lung,

iv) the small quantities of particles administered do not alter the distribution of blood flow,

v) metabolism of the particles is not so rapid as to alter the initial distribution before their detection by external radiation detectors, and

vi) proper calibration can be used to correct for the effects of variations in chest wall thickness,

lung volume and other geometrical factors.

The main types of radioactive particles used in lung scanning will be briefly discussed separately.

a) Labeled Insoluble Particles

Labeled particles were first used to localize radioisotopes in the lung in an attempt to treat cancer. Muller and Rossier (62) and later Pochin et al. (63) suggested the use of charcoal particles onto which had been adsorbed gold-198. Ariel (64) suggested the use of ceramic microspheres labeled with various radioisotopes in the diagnosis of pulmonary embolism and infarction. Insoluble particles such as these were not used extensively because of the possible adverse effects.

b) Labeled Macroaggregates of Albumin

Ever since the use of macroaggregated human serum albumin labeled with iodine-131 was first reported (65,66) this preparation has remained one of the most widely used lung scanning agents. ^{131}I-Macroaggregated human serum albumin is commercially available in a ready-to-use form from several manufacturers.

Labeled albumin macroaggregates in the size range of 10 to 70 microns is considered to be the most satisfactory clinically (67). Deland (68) found that the radioactivity (corrected for decay) of ^{131}I-macroaggregated albumin disappeared from the lung area with a half-time of six hours.

Macroaggregated human serum albumin has also been labeled with the shorter lived radioisotopes ^{99m}Tc (69,70,71) and ^{113m}In (72,73).

The use of labeled macroaggregated human serum albumin has proven to be a relatively safe procedure. It has been estimated that this procedure has been used with several hundred thousand patients (67) and only two cases where death has occurred have been reported (74,75). In both cases, preexisting pulmonary hypertension was present.

c) Labeled Iron Hydroxide Particles

Stern and associates (6) were the first to use ferric hydroxide particles labeled with ^{113m}In for lung scanning. In mice, they found that initial uptake in the lung was 85% of the intravenously administered dose. The initial clearance of ^{113m}In-ferric hydroxide particles was relatively rapid with a half-time of 15.6 hours (76). However, it has been pointed out by Barker et al. (77) that there also was a second component with a long half-time in the clearance curve. This component comprised about 10% to 20% of the dose and had a half-time of between 40 and 80 days (78,79).

Trow et al. (80) have suggested the use of ^{113m}In-labeled aluminum hydroxide particles rather than ferric hydroxide particles since the ferric ion exhibits considerable toxicity.

Technetium-99m-labeled ferrous hydroxide particles were used for the first time by Boyd and his associates (81).

These authors found that the particles had a biological half-time of about 24 hours in the lungs. Yano (82) reported that ^{99m}Tc-ferric hydroxide particles were concentrated to the extent of 85% to 87% in the lungs of rats. Davis (83) found that ^{99m}Tc-ferrous hydroxide particles could not be sterilized by terminal autoclaving as this destroyed the aggregates. Kits for the preparation of ^{99m}Tc-ferrous hydroxide particles have recently become commercially available.

3. Radioaerosols

In order to evaluate lung perfusion scans correctly, information about regional pulmonary ventillation is often necessary. For this reason radioaerosol inhalation scanning was introduced (84,85,86). This procedure involves the inhalation of aerosols composed of labeled particles ranging from 0.1 to 2.0 microns in size. A large proportion of the radioactivity is distributed evenly throughout the lower respiratory tract with very little in the upper respiratory tract in normal persons. The aerosol is produced with a positive pressure or ultrasonic nebulizer (85). Particles that have been used include ^{99m}Tc-albumin (87), ^{113m}In-albumin (88) and ^{113m}Indium chloride (89).

EXPERIMENTAL METHODS

A. Animals

Male adult mice of the ALAS strain were used for tissue distribution, excretion and toxicity studies. The mice weighed 20 - 25 g when received and were allowed food (Teklad Rockland Mouse/Rat Diet) and water ad libitum.

A female New Zealand White rabbit weighing about 3 kg and a female mongrel dog weighing approximately 13 kg were used for lung scanning studies. These animals were also allowed food and water ad libitum.

B. Preparation of Chemicals, Solutions and Suspensions

All chemicals were of ACS standard. Double distilled water was used throughout this investigation.

1. Preparation of Indium Oxinate

Indium oxinate was prepared and separated either by extraction into chloroform or by precipitation.

a) Extraction of Indium Oxinate into Chloroform

A modification of the method proposed by Moeller (15) for the quantitative extraction of indium was used. An acetate solution of indium trichloride was prepared by dissolving 250 mg of anhydrous sodium acetate, 0.25 ml of glacial acetic acid and 292 mg of indium trichloride (J.T. Baker Chemical Co., Phillipsburg, N.J.) in 25 ml of water. This solution was extracted with four 5 ml portions of a chloroform solution containing 580 mg of oxine

(J.T. Baker Chemical Co., Phillipsburg, N.J.) in 20 ml of chloroform. The chloroform extract containing indium oxide was then evaporated using a flash evaporator.

b) Precipitation of Indium Oxide

The method of Geilmann and Wrigge (32) was used to form indium oxinate by precipitation. A 3% solution of oxine was prepared by mixing 1.5 g of oxine with 2 ml of glacial acetic acid and dissolving in 40 ml of hot water. Sufficient distilled water was added to produce a total volume of 50 ml. A 10 ml portion of this oxine solution was then added dropwise to 25 ml of an aqueous acetate solution containing 146 mg of $InCl_3$, 250 mg of anhydrous sodium acetate and 0.25 ml of glacial acetic acid, causing precipitation of indium oxinate. The precipitate formed was collected by filtration.

The indium oxinate prepared by either of the above methods was purified by three successive recrystallizations from a dioxane:water (1:2) solution. The final product was dried at 120°C for one hour.

2. Analysis of Indium Oxinate Chelate

Indium oxinate samples, prepared by the above methods, were analyzed by Infra-Red Spectrophotometry (I.R.), Nuclear Magnetic Resonance Spectrometry (N.M.R.) and by Carbon-Hydrogen-Nitrogen analysis.

I.R. analyses of oxine and indium oxinate were

performed by preparing 3% solutions in chloroform. Sodium chloride cells with a pathlength of 0.5 mm were used. The I.R. spectrum of oxine was characterized by absorption bands at 3410 cm^{-1} and 3050 cm^{-1} (O-H stretching vibrations) and at 1405 cm^{-1} and 1155 cm^{-1} (phenol O-H bending and C-O stretching vibrations). These bands were not observed in the case of indium oxinate spectrum. However, with this latter compound there was an absorption band at 2960 cm^{-1} (chelate stretching vibration) (90). The respective I.R. spectra that were observed for indium oxinate and oxine are demonstrated in Figures 2a and 2b.

The N.M.R. spectrum of oxine showed a singlet at about 0.82 τ. This absorption may have been due to an intramolecular bonded hydroxyl group (90). On the other hand, there was no absorption in the region of 0.82 τ in the N.M.R. spectrum of indium oxinate.

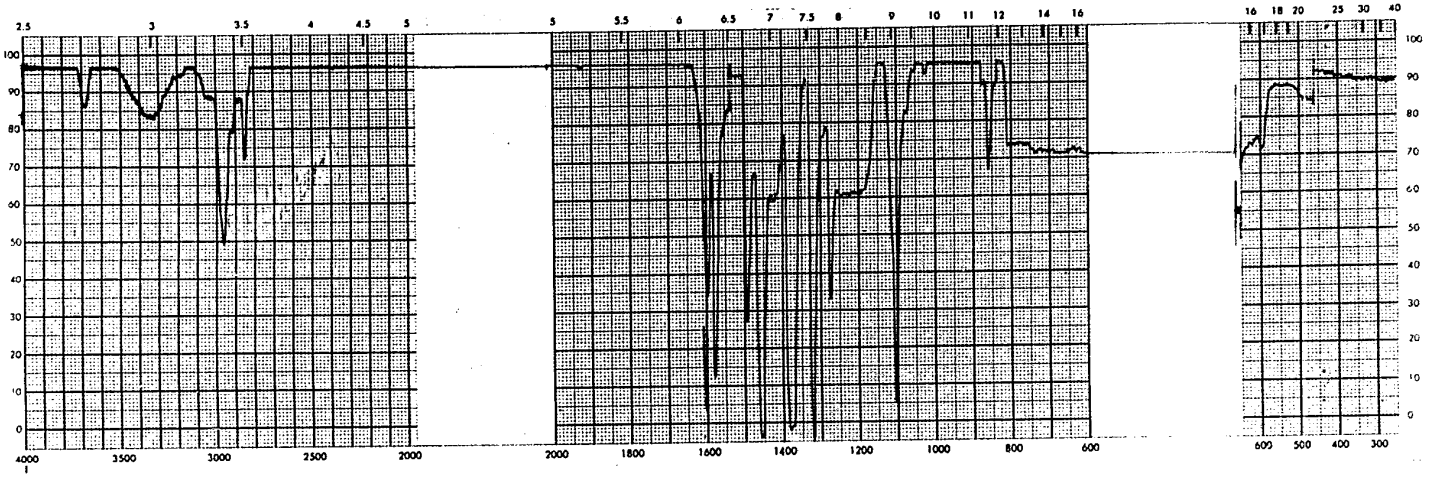
Carbon-Hydrogen-Nitrogen analysis of indium oxinate showed the presence of 58.72% carbon, 4.10% hydrogen and 7.59% nitrogen. Calculated amounts for indium oxinate are 58.93% carbon, 3.85% hydrogen and 7.64% nitrogen.

From these results it was concluded that the separated material was indium oxinate.

3. Preparation of ^{113m}In-Indium Oxinate Ethanolic Solution

The solubility of indium oxinate in water is low. Therefore, ^{113m}In-indium oxinate in a 20% ethanolic solution was prepared to study its tissue distribution. $^{113m}InCl_3$

a) Infrared Spectrum of Indium Oxinate (3% w/v in Chloroform)



b) Infrared Spectrum of Oxine (3% w/v in Chloroform)

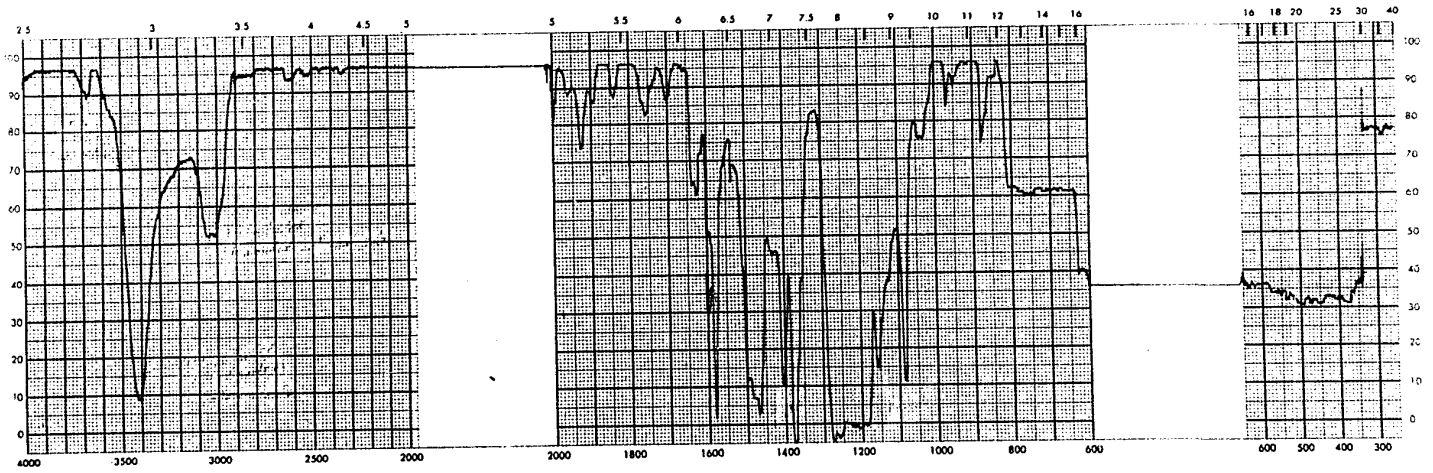


Figure 2

in 0.05% HCl was obtained from a 5 mCi ^{113}Sn-^{113m}In generator (New England Nuclear, Boston, Mass.). To two ml of the generator eluate was added, with stirring, 0.5 mg of oxine in 0.1 ml of 10% acetic acid, 0.1 mg of $InCl_3$ in 0.1 ml of 0.1N HCl, and 0.5 ml of absolute ethanol. The pH of the solution was adjusted to about 11.5 to achieve quantitative chelation and then lowered to about 7.

4. Preparation of ^{114m}In-Indium Oxinate Ethanolic Solution

Because long-term distribution studies were necessary, the longer lived isotope ^{114m}In (half-life 50 days) was used. ^{114m}In-Indium oxinate was prepared by extraction into chloroform as previously described. The stock solution of $^{114m}InCl_3$ (New England Nuclear, Boston, Mass.) contained 1.58 mg of indium per ml. An aqueous solution containing 0.25 ml of glacial acetic acid, 250 mg of anhydrous sodium acetate and 0.24 ml of $^{114m}InCl_3$ stock solution (0.38 mg indium) in 25 ml was extracted with 1.5 mg of oxine in 10 ml of chloroform and washed with two 5 ml portions of chloroform. The combined extracts and washings were evaporated. A portion (1.0 mg) of the ^{114m}In-indium oxinate thus obtained was dissolved in 2 ml of absolute ethanol. Sufficient water was added to produce a total volume of 10 ml.

5. Preparation of ^{113m}In-Indium Oxinate Suspension

The ^{113m}In-indium oxinate suspensions were prepared by adding, with stirring, to each ml of generator eluate,

0.5 mg of oxine in 0.1 ml of 10% acetic acid and 0.1 mg of $InCl_3$ in 0.1 ml of 0.1N HCl. The pH was adjusted to 11.5 with 1N NaOH and then to about 7 with 1N HCl. The preparation was autoclaved for 20 minutes at 121°C. By this procedure, about 1.5 ml of the final product were obtained for each ml of generator eluate used.

6. Preparation of ^{114m}In-Indium Oxinate Suspension

^{114m}In-Indium oxinate suspensions were prepared by adding 0.47 ml of $^{114m}InCl_3$ stock solution (1.0 mg of $InCl_3$) and 5 mg of oxine in 1.0 ml of 10% acetic acid to 10 ml of 0.05N HCl. The pH was then adjusted to 11.5 with 1N NaOH and then to 7 with 1N HCl. The suspensions were autoclaved for 20 minutes at 121°C. The final volumes of the preparation were about 15 ml.

7. Determination of Chelation of Radioactive Solutions and Suspensions

Each batch of labeled solution or suspension of indium oxinate was checked for completeness of chelation by chromatography on Whatman No. 1 paper using chloroform as solvent. Radioactive indium chloride (acidic), indium hydroxide, and indium chloride in acetate were found to remain at the origin, while labeled indium oxinate moved on the paper chromatogram with a radioactive peak at the solvent front. A batch of suspension or solution was considered acceptable if less than 2% of the total activity remained at the spot of origin.

C. Tissue Distribution Studies

The tissue distribution of the indium oxinate solutions or suspensions were studied in mice. The radioactive solution or suspension being investigated was injected *via* the tail vein at the rate of about 0.02 ml per second. At specified time intervals after injection the animals were sacrificed by decapitation. A sample of blood was immediately collected and measured using a 1 ml syringe. The sample was then transferred directly into a counting tube. The entire heart, lungs, liver, brain, kidneys and spleen were excised and samples of muscle and fat tissue were taken. The tissues and organs were blotted free of blood, weighed and transferred to counting tubes.

At the time of injection, a sample of the solution or suspension equivalent to the dose administered to the animals was put into a counting tube. This was counted at the same time as the tissues and used as a standard for the dose administered. Thus the need for corrections for physical decay of the isotope was eliminated.

The organs and tissues were counted for one minute in a Picker Autowell II gamma spectrometer (Picker Corporation, Cleveland, Ohio). Tissues were counted without wet ashing as this was found to produce count rates not statistically different than those observed using the present technique. The standard dose was counted without dilution as there was found to be less than 1% loss in count rate when 0.05 ml of the ^{114m}In-$InCl_3$ solution was diluted to a three milliliter

volume.

In all cases the tail of the animal was counted to ascertain whether any appreciable amounts of radioactivity remained at the site of injection.

1. Tissue Distribution of ^{113m}In-Indium Oxinate Ethanolic Solution

In a preliminary tissue distribution of ^{113m}In-indium oxinate ethanolic solution nine mice were each injected intravenously with 0.1 ml of a solution containing about 30 μCi of radioactivity. Three mice were sacrificed at one, six and twelve-hour intervals after injection. Samples of the blood, various organs and tissues were counted and the radioactivity expressed relative to the standard dose.

2. Tissue Distribution of ^{114m}In-Indium Ethanolic Solution

A ^{114m}In-indium oxinate solution was prepared as previously described. In a preliminary study, mice were injected intravenously with 0.1 ml (4 μCi) of the solution three days after its preparation. Three animals were sacrificed at each of 15 minutes, 6 hours, 24 hours and 72 hours after injection. The tissue distribution of the activity was compared to the standard dose.

3. Tissue Distribution of ^{114m}In-Indium Oxinate Suspension

Mice were injected with 0.05 ml (5 μCi) of ^{114m}In-indium oxinate suspension. Five mice were sacrificed at each of 10 minutes, 3 hours, 6 hours, 12 hours, 18 hours,

3 days, 7 days, 14 days and 21 days after injection. Tissue radioactivity was measured and compared to the standard dose.

4. Tissue Distribution of ^{113m}In-Indium Oxinate Suspension

Six batches of ^{113m}In-indium oxinate suspension were prepared. Of each batch, 0.1 ml was injected into a mouse. Each dose contained about 10 μCi of radioactivity at time of injection. Six mice were then sacrificed 15 minutes after injection and a tissue distribution of the radioactivity determined.

D. Whole Body Excretion Analysis of Indium Oxinate Suspension

1. Whole Body Counting

Five mice were each administered 0.05 ml (5 μCi) of ^{114m}In-indium oxinate suspension by tail vein injection. This contained about 8 μg of indium oxinate and 10 μg of oxine per dose. At various time intervals after injection the animal was placed in a plastic bottle 5 inches long and 2.2 inches in diameter which was then lowered into a small animal whole body counter. The radioactivity was detected by two 3" X 3" NaI(Tl) crystals and recorded.

The animals were counted 5 minutes, 30 minutes, 2 hours and 4 hours after injection and then daily for 35 days. The count observed 5 minutes after injection was considered as that due to the dose injected into the animal. All subsequent counts were corrected for physical decay back

to that time.

The effect of geometry on the count rate in the whole body counter used was investigated by Lyster (92). A model of a mouse was prepared and a radioactive source was placed in various positions in the model. The model was placed in various positions in relation to the NaI(Tl) crystals. Counts were taken at each of these various points. The standard deviation of the counts was found to be small. At certain positions of the source the detection of radioactivity was about 18% less than at others. However, it was felt that these positions represented extreme conditions and would not normally exist.

2. Compartmental Analysis

As mentioned previously, the counts obtained for each animal at 5 minutes after injection were used as representing the initial dose to the animal. Radioactivity observed at various times after this was corrected for decay and expressed as a percentage of the initial activity. The mean and standard deviation of the five values (one for each animal) at each time interval was then calculated. These means were used to plot the excretion curve.

The excretion curve may be broken into a series of exponential components. Assuming that these exponents are separate and unrelated, the activity remaining in the body

at time "t" is given by the equation (92)

$$f(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i t}$$

where A_i is the activity of the ith component at zero time; λ_i is the elimination rate constant of the ith component; t is the time after administration.

In biological systems the exponent components are not separate and unrelated. In this study, however, no corrections were made for these factors.

Dick and Lea (93) have presented a method whereby the last portion of the excretion curve can be analyzed to determine if it is composed of a single exponential component or an exponential component plus one or more other exponentials or a bound fraction. The rate of change can be represented as

$$\frac{dA_i}{dt} = -\lambda(A_i - A_B) = -\lambda A_i + \lambda A_B$$

where A_i is the fraction of the activity in the exponential component and A_B is the fraction of the activity bound or in other exponential components.

Therefore, when $dA_i/dt = 0$, $A_i = A_B$. By plotting on linear graph paper the rate of change (dA_i/dt or Percent Loss per Time Interval) versus the amount remaining (A_i or Percent Remaining) at the beginning of the time period for the last portion of the excretion curve a straight line is obtained.

The intercept of this line on the "Percent Remaining" axis represents the activity remaining when the rate of change is 0. If the line passes through the origin it can be assumed that the last exponential on the curve represents all remaining radioactivity. If the line passes through some other point on the "Percent Remaining" axis, this point represents activity that is not represented by the last exponential component of the curve. The value of this radioactivity must then be subtracted from all data and the excretion curve replotted.

In order to resolve the exponential components of the excretion curve obtained, a digital PDP 8/L computer (Digital Equipment Corporation, Maynard, Mass.) was used. The program used calculated a line of best fit using a weighted least squares method. The weighting factor consisted of a weight allowing for the log ordinate plus a weight allowing for the statistical variation of the data. Data representing the final three points on the excretion curve were fed into the computer. The line of best fit was calculated. Data corresponding to the following point were then fed in. A new line was then fitted to all four points. The slope of this line was compared to the original line by means of a Students 't' test. The 't' value and its degree of freedom were printed out. This process was continued until a statistical difference occurred between the slopes of 'n' and 'n + 1' points. The computer was then instructed to use 'n' points. The computer calculated the half-time of

the slope, the standard deviation of the half-time and the ordinate intercept value. This information was used to correct the remaining data for the contribution of the component having the longest half-time. Using the new data the process was repeated. This continued until no more data remained.

3. Route of Excretion of Indium Oxinate Suspension

Five mice were injected with 0.05 ml (5 μCi) of ^{114m}In-indium oxinate suspension. These animals were housed individually in metabolism cages. The urine and feces were collected at 21 days, evenly distributed among a number of counting vials and counted in a Picker Autowell II. The total counts were compared to a standard dose.

An attempt was made to extract the radioactivity from the urine and feces with chloroform. In addition, paper chromatography was attempted using both chloroform and water and ethanol (1:1) as solvents in an effort to identify the metabolites in the urine.

E. Toxicity Studies

1. Toxicity of Indium Oxinate Particles

It was found that stable indium oxinate which had been precipitated from dioxane-water had approximately the same particle size as that of the particles in the ^{113m}In-indium oxinate suspensions. Toxicity studies on mice were carried out by intravenous injections of various concen-

trations of indium oxinate particles in suspension.

a) Lethal Doses

Various concentrations of indium oxinate suspension in 2% w/v polyvinylpyrrolidone (PVP) were prepared. The use of the suspending agent was necessary in view of the fact that a large number of particles were present in the suspensions. The concentrations of the suspensions were varied in order that a constant volume (0.01 ml per g body weight) could be administered at the various dose levels used. All suspensions were autoclaved at 121°C for 20 minutes before administration.

Doses of 50, 100, 125 and 150 mg of indium oxinate per kg were administered to groups of mice. Any deaths which occurred did so within 12 hours. However, surviving animals were maintained for at least one month in order to detect any long term effects.

b) Effect on Weight Gain

Five animals, weighing 20 - 23 g, were administered indium oxinate particles intravenously at a dose of 100 mg per kg. These animals were weighed at various times up to 40 days after indium oxinate administration. The weights of these animals were compared to those of controls at each time period.

c) Histopathological Effects

Histopathological studies were done on the lung, liver

and kidneys of mice that were sacrificed 1, 17 and 48 days after intravenous injection of indium oxinate at a dose of 100 mg per kg.

2. Toxicity of Oxine Particles

Crystals of oxine were reduced mechanically to a size that approximated that of the ^{113m}In-indium oxinate particles (10 to 30 microns). Suspensions containing various concentrations of oxine in 2% w/v were prepared. The concentrations were adjusted in order to keep the volume administered constant. The suspensions were prepared under aseptic conditions, but terminal autoclaving was not used since oxine has a low melting point.

Doses of 40, 50, 75 and 100 mg of oxine per kg body weight were administered to groups of mice. Although deaths were confined to the initial 24 hour post-injection period, surviving animals were observed for at least one month.

F. Scans

A Picker Magnascanner 500/D rectilinear scanner (Picker Corporation, Cleveland, Ohio) was used to obtain lung scans. A scanning speed of 20 cm per minute was used. Background supression and contrast enhancement were set at a minimum.

A female New Zealand White rabbit weighing approximately 3 kg was tranquillized with an intramuscular dose of 0.3 ml of Innovar-Vet (0.4 mg fentanyl and 20 mg droperidol per milliliter, McNeil Laboratories, Don Mills, Ontario). Approximately 300 μCi of ^{113m}In-indium oxinate was administered *via* the ear vein. The animal was positioned on its back and scanning was begun immediately.

A lung scan of a female mongrel dog weighing about 13 kg was also obtained. The dog was tranquillized with 1.5 ml of Innovar-Vet and 0.25 ml of atropine (0.4 mg per ml). About 800 μCi of ^{113m}In-indium oxinate suspension was injected intravenously. Scanning was begun after positioning the dog on its back.

G. Quality Control

1. Radiochemical Purity

a) Indium-114m

For long term distribution and excretion studies, the longer lived radioisotope, indium-114m (half-life 50 days), was used. Indium-114m was obtained as indium trichloride in 2N HCl from New England Nuclear, Boston, Mass. The specific activity of the stock solution was 1.26 mCi per mg.

The radiochemical purity of the ^{114m}In-indium trichloride stock solution was confirmed by its energy spectrum. A sample of the stock solution was placed in

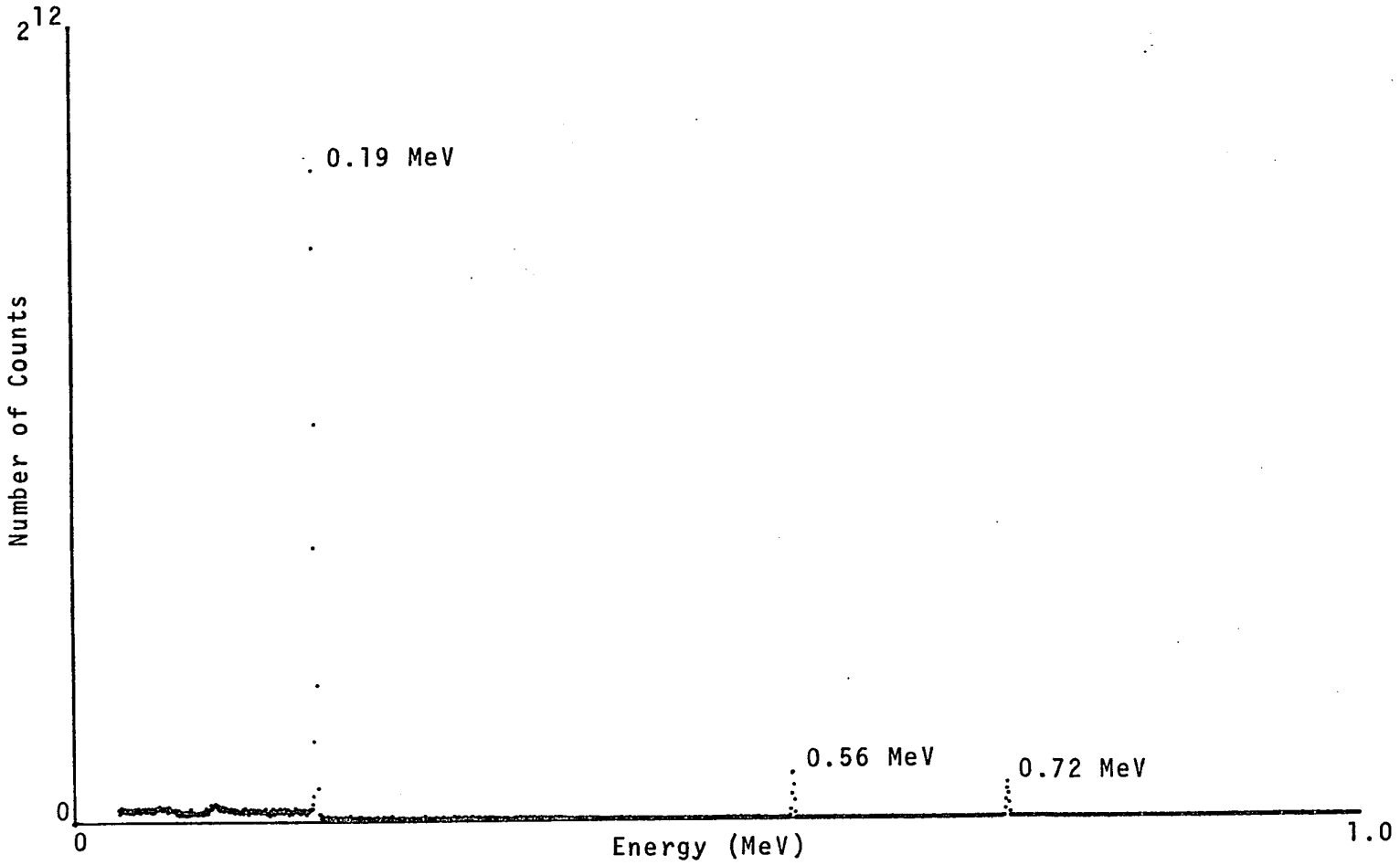
front of a Ge(Li) detector (Nuclear Diodes, Prairie View, Ill.). The energy spectrum was stored in a multichannel analyzer (Northern Scientific, Middleton, Wis.) and then plotted using an X-Y plotter. The resulting spectrum is shown in Figure 3. Peaks were seen at 0.19 MeV, 0.56 MeV and 0.72 MeV. These agree with published gamma emissions of ^{114m}In (1). As no other peaks were evident the radiochemical purity of the ^{114m}In-indium chloride stock solution was acceptable.

b) Indium-113m

Indium-113m was obtained by eluting a 5 mCi ^{113}Sn-^{113m}In generator (New England Nuclear, Boston, Mass.) with 0.05N HCl. When the generator was first received, 8 ml of eluate contained about 4 mCi of ^{113m}In as determined with an isotope calibrator (Nuclear Associates, Westbury, N.Y.) and by comparison with a Ba-133 standard. Indium-113m obtained from a generator is essentially carrier free.

The generator-produced ^{113m}In was checked for radiochemical purity by determination of the gamma energy spectrum, by determination of half life and by analysis for tin-113 breakthrough.

A sample of the generator eluate was placed in front of the Ge(Li) detector and an energy spectrum obtained using the multichannel analyzer. The energy spectrum that



Energy Spectrum of Indium-114m Determined Using a Ge(Li) Detector

Figure 3

was obtained is shown in Figure 4. Only one peak was observed. The energy of this gamma emission (0.39 MeV) agrees with the published values for ^{113m}In (1).

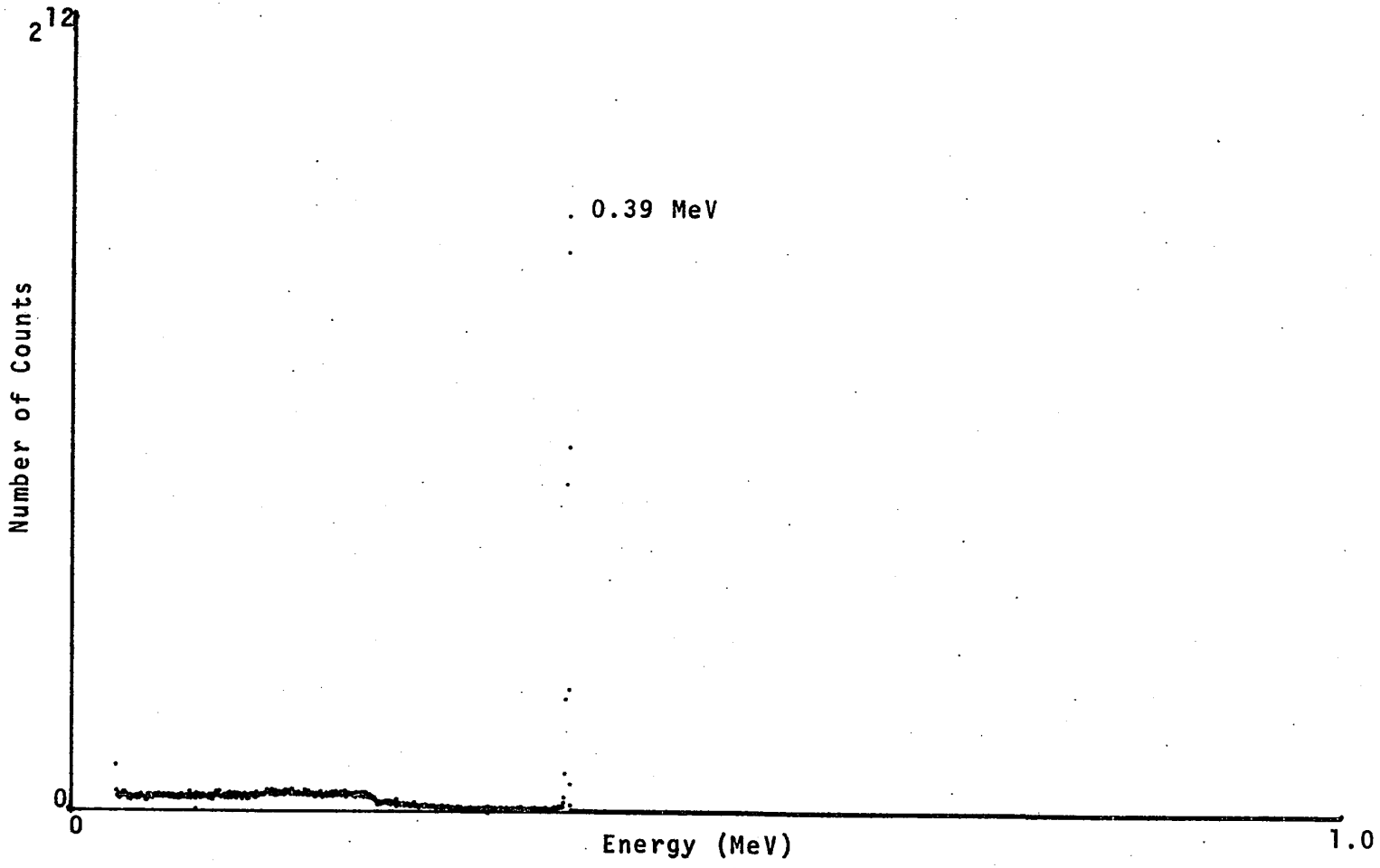
The half-life of the generator-produced ^{113m}In was determined by counting a sample of the eluate for one minute every 24.5 minutes for a total time of 392 minutes. The decay was found to have a half-time of 100.3 ± 0.2 minutes. Published values for the half-life of ^{113m}In range from 99.8 minutes to 103 minutes (1).

The breakthrough of tin-113 from the generator column was determined on several different occasions using the method supplied by the manufacturer (102). This method entails allowing the eluate to decay for at least 48 hours. Any activity that is present in a sample of the eluate after this time is assumed to be due to ^{113m}In in equilibrium with ^{113}Sn. By comparing the activity present to that of a ^{133}Ba standard, the amount of ^{113}Sn present is determined. At no time was tin-113 found to account for more than 0.01% of the total eluted activity.

From these results it was concluded that the generator eluate was essentially radiochemically pure.

2. Particle Size of Indium Oxinate in Suspension

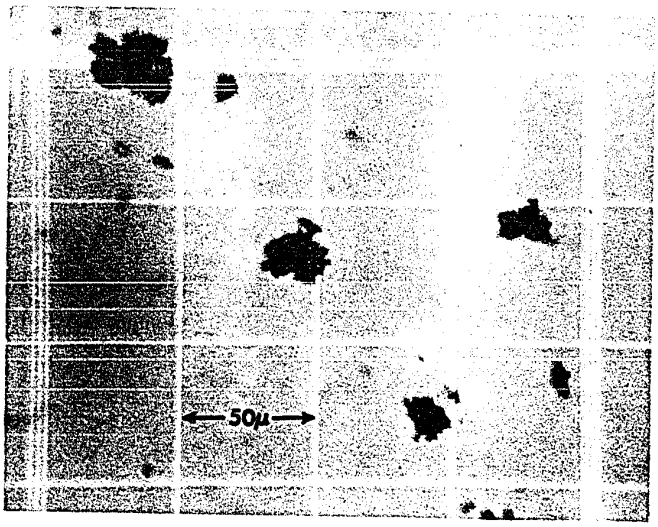
As determined by a hemacytometer, each milliliter of the ^{113m}In-indium oxinate suspension contains approxi-



Energy Spectrum of Indium-113m Determined Using a Ge(Li) Detector

Figure 4

mately 500,000 particles in the size range of 10 to 30 microns. There is only a small percentage of the particles smaller than 10 microns. In numerous batches that were prepared no particles greater than 50 microns in diameter were observed. Figure 5 is a photograph of a sample of ^{113m}In-indium oxinate particles as seen under a microscope.



^{113m}In-Indium Oxinate Particles

Figure 5

In an attempt to further characterize the particle size of the indium oxinate in suspension a Coulter Counter (Coulter Electronics, Hialeah, Fla.) was used. The results obtained by this method indicated that in each milliliter

of suspension there were about 560,000 particles greater than 9.38 microns in diameter. No particles greater than 37.5 microns in diameter were recorded. Of all particles greater than 1 micron in diameter, about 78% by weight were found to be greater than 9.38 microns.

Measurement of particle size using a Coulter Counter is based on volume. The diameter is calculated from the volume assuming that the particles are spheres. As can be seen from Figure 5, the indium oxinate particles are not spheres. Therefore, this method will tend to indicate that the diameter of a particle is less than it actually is. As the indium oxinate particles are not of a regular shape, no attempt was made to correct for this factor.

RESULTS AND DISCUSSION

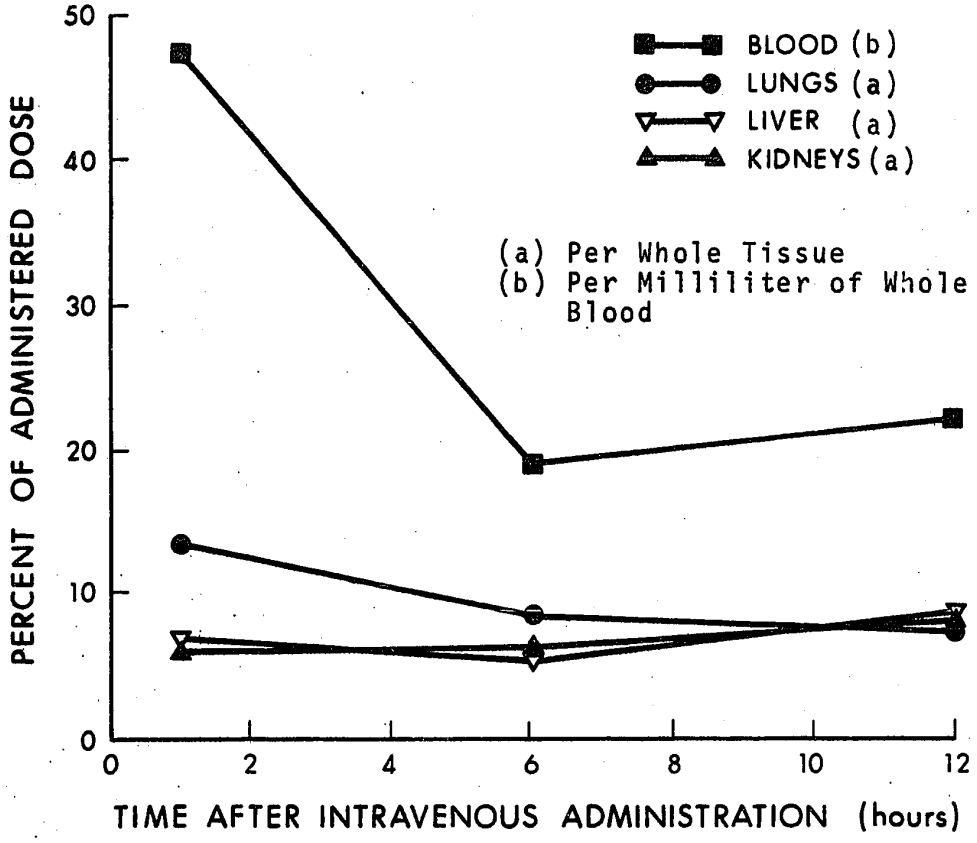
A. Tissue Distribution in Mice

1. ^{113m}In-Indium Oxinate Ethanolic Solution

A preliminary tissue distribution study of ^{113m}In-indium oxinate solution was carried out in groups of three mice at intervals of 1 hour, 6 hours and 12 hours after intravenous injection. The results of this study are shown in Appendix 1 (Table 1). Figure 6 shows the mean uptake in the major sites of distribution at the various times. It appeared that, initially, a major proportion of the radioactivity remained in the blood. Radioactivity found in other tissues and organs was probably largely due to the presence of blood since the organs were not perfused.

2. ^{114m}In-Indium Oxinate Ethanolic Solution

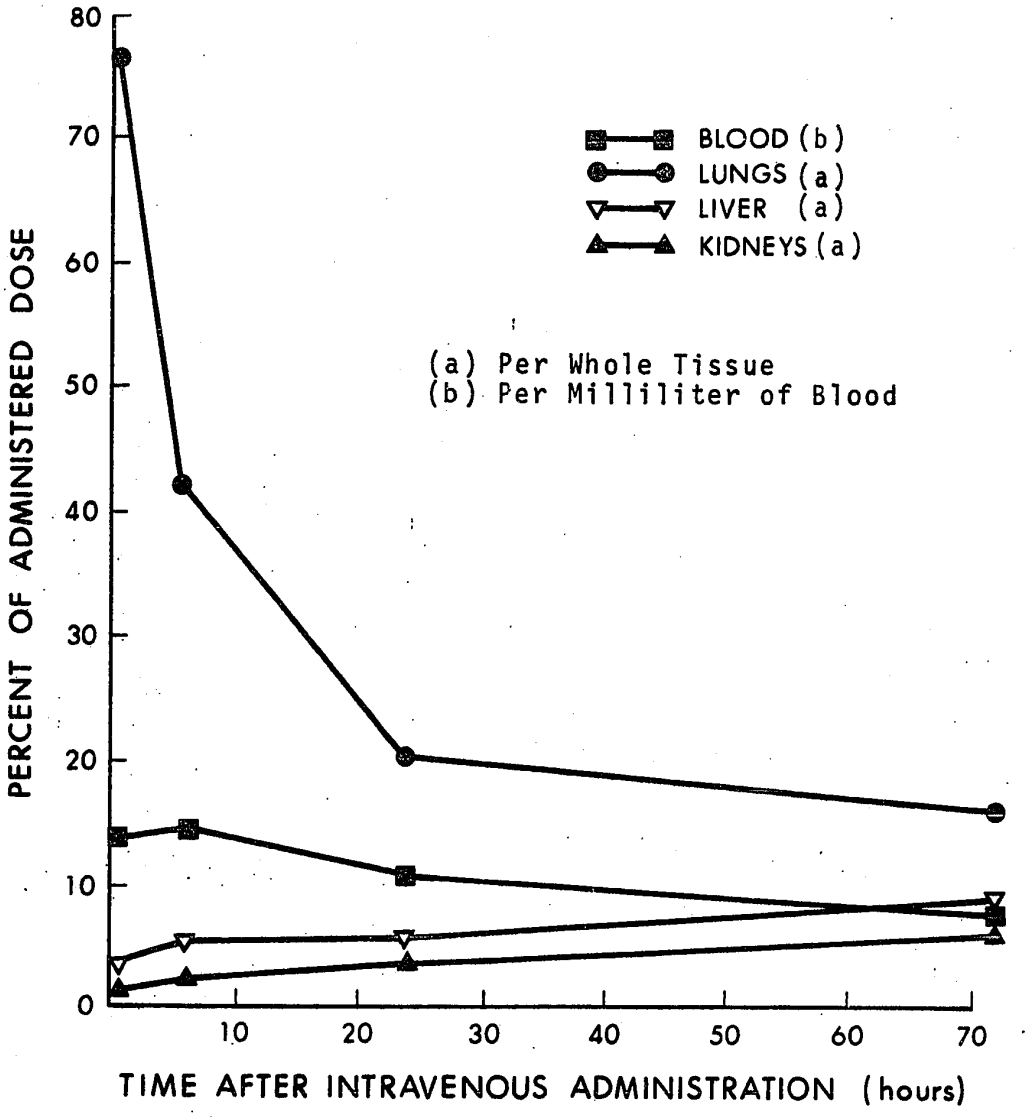
In order to determine if any delayed uptake of ^{114m}In-indium oxinate occurred, a preliminary longer term distribution study of ^{114m}In-indium oxinate was carried out. Figure 7 shows the results of this study for the major sites of uptake. The mean and standard deviation of each organ and tissue studied is shown in Appendix 1 (Table 2). These results differed greatly from those using ^{113m}In-indium oxinate solution. In this study a high initial concentration of radioactivity was noted in the lung. This was believed to have been caused by the precipitation of indium oxinate upon injection. The radioactive solution had been prepared several days before use and it may have been close



Tissue Distribution

of ^{113m}In-Indium Oxinate Solution in Mice

Figure 6



Tissue Distribution

of ^{114m}In-Indium Oxinate Solution in Mice

Figure 7

to the saturation point. Dilution of the ethanolic solution by the blood may have brought about precipitation. Geilmann and Wrigge (32) report the solubility of indium oxinate to be 0.002% in 10% ethanol and 0.057% in 50% ethanol. The concentration of the solution used in this study was 0.01% indium oxinate in 20% ethanol and thus it may have been near saturation.

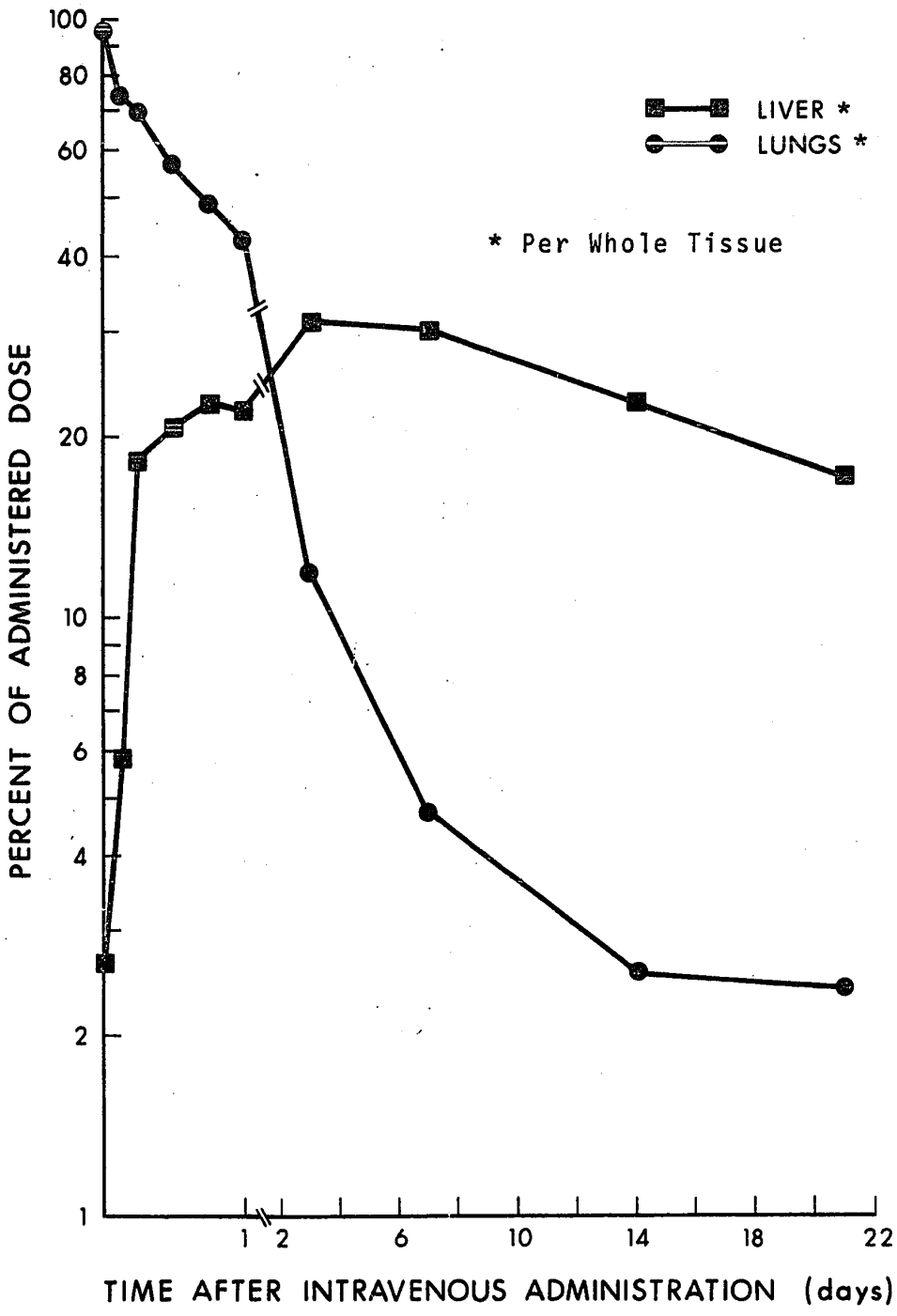
The results of this preliminary investigation suggested the possibility of the use of ^{113m}In-indium oxinate particles for lung scanning purposes.

3. ^{114m}In-Indium Oxinate Suspension

Tissue distribution studies were done using five mice at each of various time periods up to 21 days after injection of 0.05 ml of ^{114m}In-indium oxinate suspension. Results are shown in Appendix 1 (Table 3) and in Figures 8, 9 and 10.

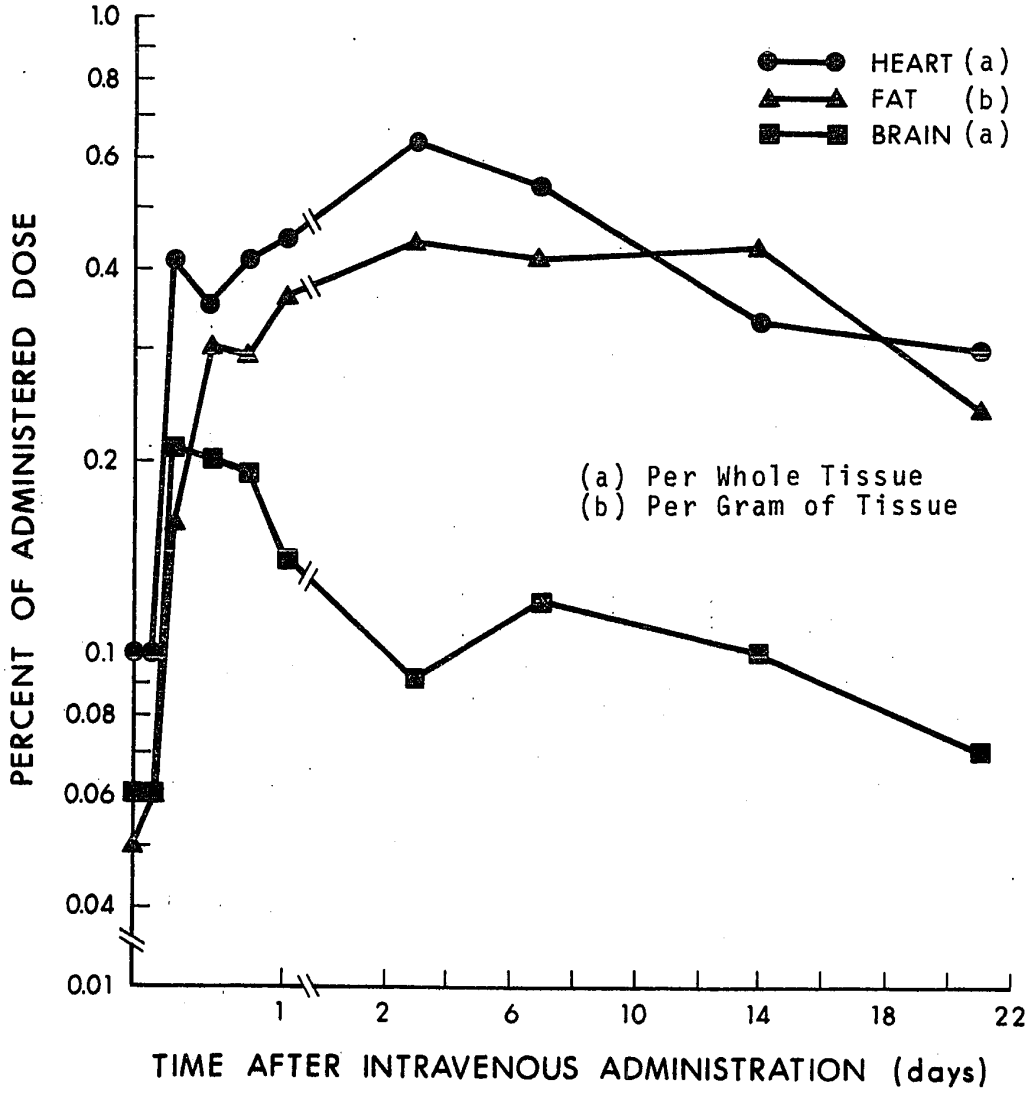
The lungs and liver were the only tissues in which a significant concentration of radioactivity occurred in the first three hours. Initially, 95% of the radioactivity was found in the lungs. As the radioactivity was cleared from the lungs it was accumulated in other tissues such as the liver, kidneys, spleen and muscle, and in the blood.

The levels of radioactivity in these tissues reached a maximum in three to seven days and then began to fall. Only a very low concentration of radioactivity was seen in



Uptake of
^{114m}In-Indium Oxinate Suspension in Lungs and Liver of Mice

Figure 8

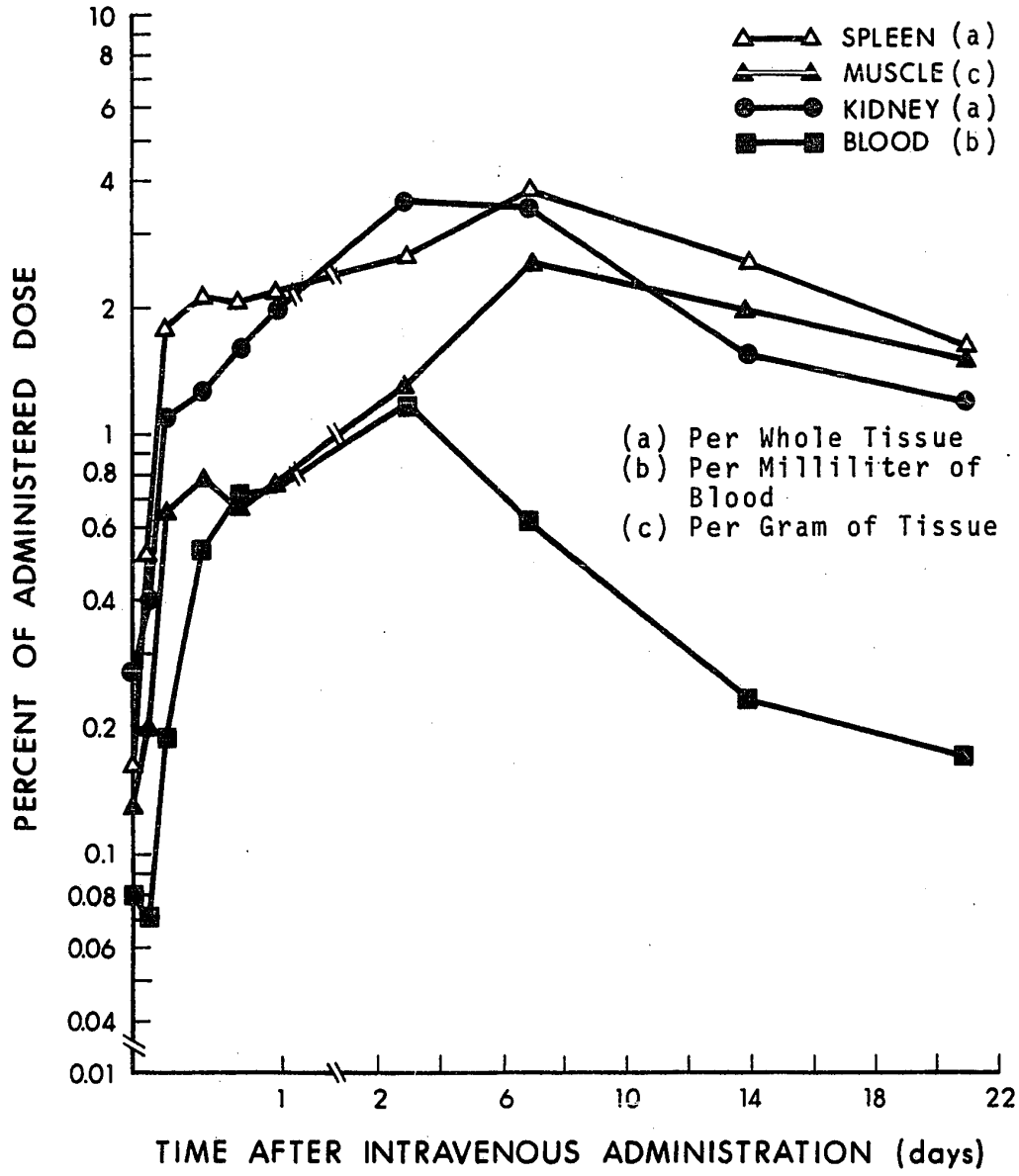


Uptake of

^{114m}In-Indium Oxinate Suspension

in Heart, Fat and Brain of Mice

Figure 9



Uptake of

^{114m}In-Indium Oxinate Suspension

in Spleen, Muscle, Kidney and Blood of Mice

Figure 10

the heart, brain and fat at any time.

The initial uptake of radioactivity in the lung was similar to that reported for currently used lung scanning agents. Stern and associates (6) reported the initial uptake of ^{114m}In-ferric hydroxide to be about 85% in the lungs of mice. The lungs of rats were found to contain 85.1% of the radioactivity ten minutes after injection of ^{99m}Tc-macroaggregated albumin (94).

The results (Figure 8) indicate that more than 20% of the radioactivity in the lung was removed within the first three hours. From three hours to three days after injection the clearance of radioactivity was exponential with a half-time of 26.1 hours. This half-time in the lung was somewhat longer than that reported for currently used lung scanning agents. ^{113m}In-Ferric hydroxide particles have an initial half-time in the lungs of 15.6 hours (76). The lung clearance half-time of ^{99m}Tc-ferrous hydroxide is about 24 hours (81). However, the clearance of ^{113m}In-ferric hydroxide particles has been reported to have a long-lived component consisting of 10% to 20% of the dose with a half-time of between 40 and 80 days (78,79). The present results showed that only about 2.5% of the dose of ^{114m}In-indium oxinate remained in the lung after 21 days. Longer term studies would be necessary to determine the rate of clearance of this remaining activity.

Of interest was the finding that suspensions of

^{114m}In-indium oxinate prepared longer than 24 hours before injection had a slower clearance from the lung than did freshly prepared suspensions. For example, an 18-hour tissue distribution of a ^{114m}In-indium oxinate suspension that was prepared about 48 hours before injection showed a concentration of 66.02 ± 4.94% of the dose in the lungs. This compares to 49.30 ± 5.87% of the dose remaining in the lungs 18 hours after administration of a freshly prepared ^{114m}In-indium oxinate suspension. This aspect would not influence the possible utility of ^{113m}In-indium oxinate as the preparation would be used almost immediately after preparation due to the short physical half-life of ^{113m}In. However, it may give a clue as to the varying results that have been observed in long term distribution studies of ^{113m}In-ferric hydroxide particles (77,78,79).

B. Characteristics and Reproducibility of ^{113m}In-Indium Oxinate Suspension

The procedure for preparation of ^{113m}In-indium oxinate suspension was previously described. This suspension contains 300 micrograms of oxine and 250 micrograms of indium oxinate (52 micrograms of In^{+3}) per ml of generator eluate (about 1.5 ml of final product).

Although it has been reported (15) that a chloroform solution of oxine will quantitatively extract indium from an aqueous solution at a pH of 3.2 to 4.5, it was found that increasing the pH to about 11.5 was necessary for quanti-

tative chelation. After the complete preparation of each batch of suspension, chelation was checked by paper chromatography using chloroform as the solvent. This method was not very definitive as the radioactivity in the chelated form followed the solvent front. However, the three other possible chemical forms of indium in the suspension, indium trichloride, indium acetate and indium hydroxide, were found to remain at the spot of origin.

The use of double distilled water was necessary in preparing the suspension. Extraneous metal cations that may be present can react with the oxine before it chelates with the indium. Even if sufficient excess oxine is present for chelation of the indium, the presence of unwanted metal oxinates may tend to retard the clearance of the particles from the lung.

Although indium oxinate has a very low solubility in water, it was found that quantitative precipitation required a considerable time at room temperature. However, after heating, the rate of precipitation increased considerably. Thus, terminal autoclaving was necessary for the complete precipitation of indium oxinate as well as to sterilize the product.

To check the reproducibility of this method for preparation of ^{113m}In-indium oxinate particles, six batches of the suspension were prepared. One-tenth of a milliliter of each suspension was injected intravenously into a mouse.

The fifteen-minute tissue distribution of radioactivity observed in these mice is presented in Table I. All six batches gave similar tissue distributions.

C. Route of Excretion of ^{114m}In-Indium Oxinate

In order to determine the route of excretion of the radioactivity, five mice were injected intravenously with 0.05 ml of ^{114m}In-indium oxinate suspension. The animals were housed individually in metabolism cages for 21 days. During this time the urine and feces were collected, divided into a number of portions and transferred into counting tubes. The total amount of excreted radioactivity was determined. The mean and standard deviation of the radioactivity found in the urine and feces of the five animals were calculated.

It was found that the urine contained 10.8 ± 4.1% of the radioactivity in the injected dose. The feces contained 19.1 ± 1.9% of the dose. Thus, approximately 30% of the dose was recovered in the urine and feces.

In order to determine if the indium was excreted as the oxinate or in some other chemical form an attempt was made to extract the urine and feces with chloroform. It was not possible to extract any significant levels of radioactivity from either the urine or the feces.

Urine samples were spotted on Whatman No. 1 paper and chromatography was attempted using both chloroform and water:

TABLE I

FIFTEEN MINUTE TISSUE DISTRIBUTION OF SIX BATCHES OF ^{113m}In-INDIUM OXINATE SUSPENSION IN MICE[b]

Tissue	Percent of Injected Dose[a]
Lungs	93.09 ± 2.18
Liver	3.56 ± 0.74
Blood[c]	0.22 ± 0.20
Spleen	0.27 ± 0.08
Kidneys	0.38 ± 0.22
Brain	0.06 ± 0.03
Muscle[d]	0.13 ± 0.05
Fat[d]	0.04 ± 0.03
Heart	0.15 ± 0.09

[a] Mean of six animals ± standard deviation

[b] Percent of dose in total organ unless otherwise indicated

[c] Percent of dose per ml of blood

[d] Percent of dose per g of tissue

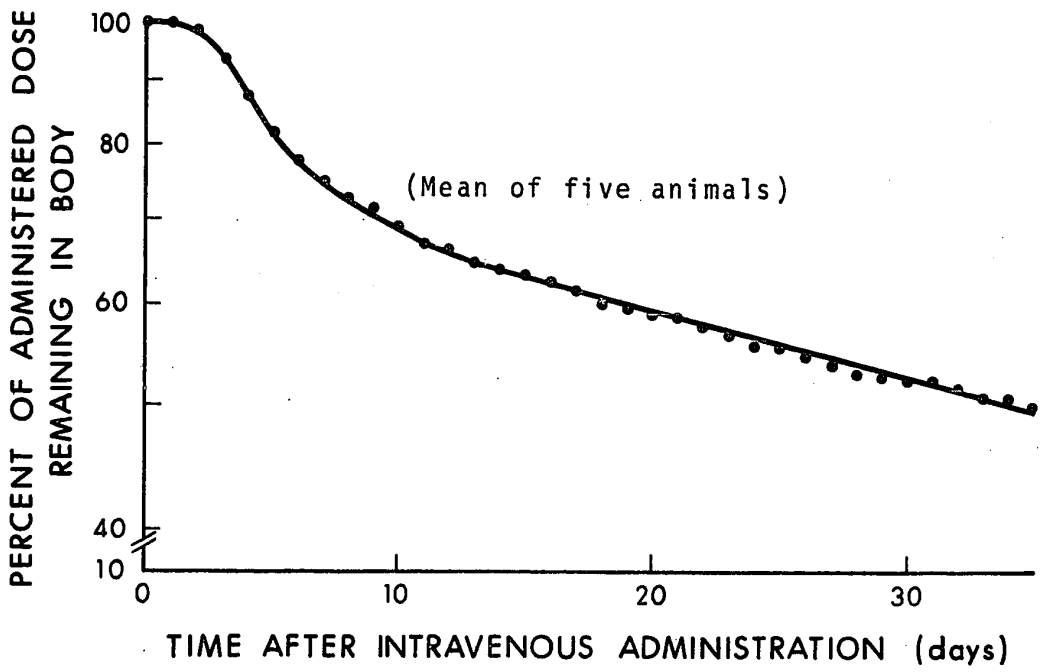
ethanol (1:1) as the solvent. In both instances all of the radioactivity remained at the spot of origin.

From these results it appeared that the indium oxinate chelate was converted in the body to some other chemical form that is insoluble in chloroform.

D. Whole Body Excretion Analysis

In order to evaluate the possibility of indium accumulation in the body after repeated use of indium oxinate, the rate of excretion of indium administered as a suspension of the oxinate in mice was determined. Five minutes after the intravenous injection of 0.05 ml of ^{114m}In-indium oxinate suspension the radioactivity in each mouse was determined using a small animal whole body counter for a period of 20 seconds. Counting was repeated 30 minutes, 2 hours and 4 hours from time of injection and then daily for 35 days. The count obtained for each individual animal at five minutes post-injection was considered as the standard initial radioactivity of that animal. All subsequent counts were corrected for decay and expressed as a percentage of the five-minute count.

The mean and standard deviation of the percentage of radioactivity remaining at each time period was calculated from the individual results of the five animals. These are shown in Figure 11 and Appendix 1 (Table 4). Since there was virtually no excretion for two days after injection,



Body Burden of
^{114m}In in Mice After Administration
of ^{114m}In-Indium Oxinate Suspension

Figure 11

only the daily results are shown.

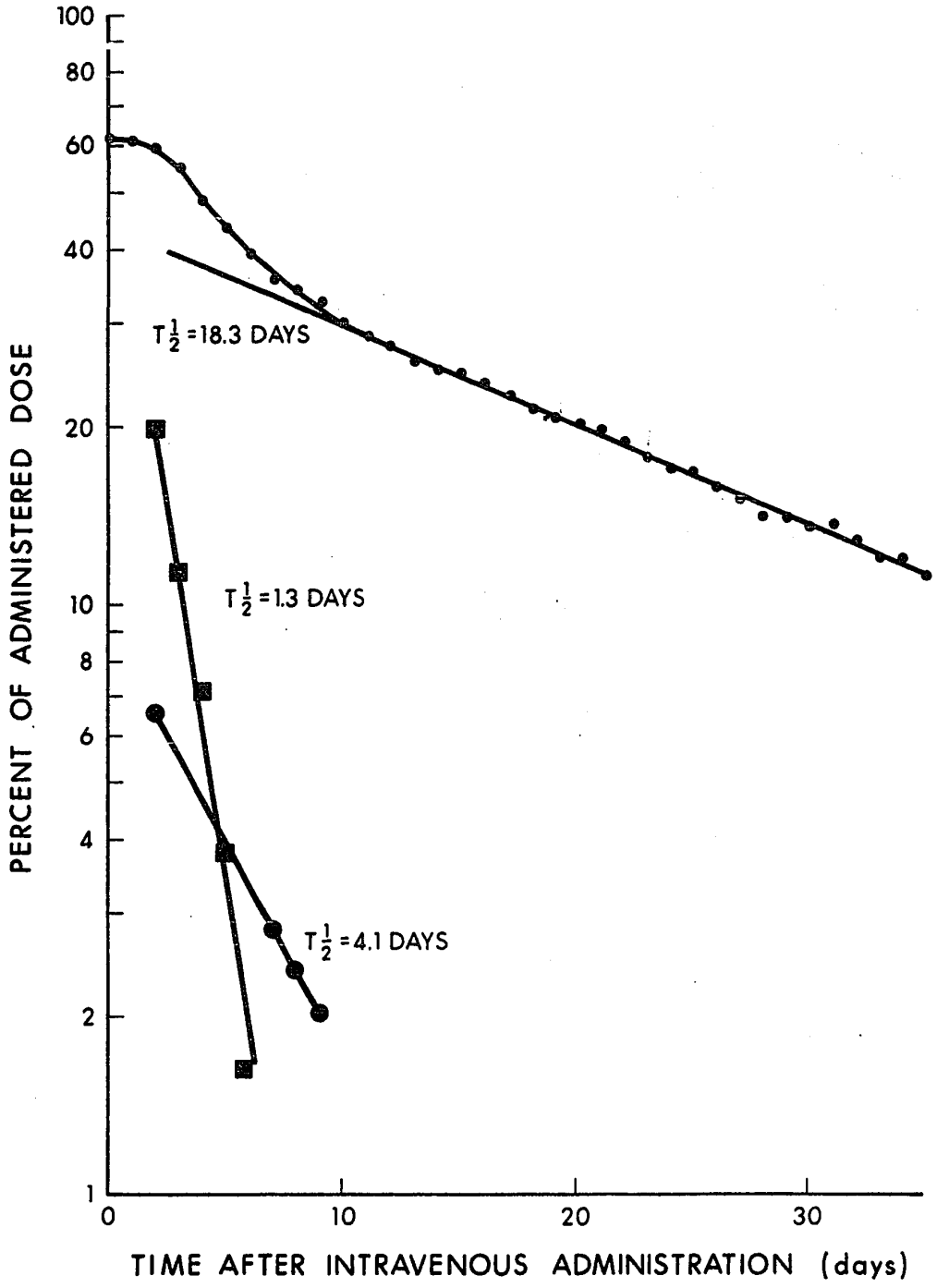
The method of Dick and Lea (93) was used to determine if the last exponential component of the excretion curve represented the radioactivity remaining in the animals when the study was ended. The rate of excretion (percent of initial radioactivity lost per day) was calculated for the last 25 points on the curve. This percent radioactivity lost per day was plotted on linear graph paper versus the percent of radioactivity remaining on that particular day. Using a PDP 8/L computer and a program for computing the unweighted least squares fit, a straight line was drawn through the points. By extrapolation of the line it was found that when the rate of excretion due to this component became zero, 38.6% of the initial radioactivity remained. Therefore, the last exponential component of the excretion curve up to 35 days post-injection did not appear to represent the final 38.6% of the radioactivity. This remaining portion of the radioactivity may have been bound in the body and excreted at a very slow rate.

For the purposes of resolving the excretion curve into separate components it was assumed that the final 38.6% of the radioactivity was bound or excreted at such a slow rate as to not affect greatly the resolution of the other components. Therefore, it was subtracted from the original data and the curve redrawn. Using the computer program previously described, this new excretion curve was resolved

into components (Figure 12). By this method the excretion curve appeared to have three components with half-times of 1.3 ± 0.1 days, 4.1 ± 0.1 days and 18.3 ± 0.3 days, respectively.

Since the excretion of radioactivity was not appreciable until the second day, the individual components were extrapolated back to that time in order to determine the fraction of radioactivity represented by each component. Consequently, it was found that the short-timed component, with half-time of 1.3 ± 0.1 days, represented 19.7% of the administered radioactivity, the component with a half-time of 4.1 ± 0.1 days represented 6.6% of the radioactivity and the component with a half-time of 18.3 ± 0.3 days represented 39.8% of the radioactivity. A large fraction of the radioactivity (38.6%) is not represented by these exponential components and is either bound or excreted at a slower rate.

The loss of radioactivity from the liver during the interval of 7 to 21 days after the administration of ^{114m}In-indium oxinate was exponential (Figure 8). This exponential component had a half-time of 17.2 days. Upon extrapolation to the time of injection it was found that this component accounted for 39.8% of the administered dose. Thus, it appears that the component of the excretion curve which had a half-time of 18.3 ± 0.3 days and represents 39.8% of the activity corresponded to the loss of radioactivity from the liver.



Compartmental Distribution of Whole Body Burden Curve of ^{114m}In After Administration of ^{114m}In-Indium Oxinate Suspension Into Mice

Figure 12

The rate of excretion of indium compounds has been studied to some extent. Smith and co-workers (19) found that the excretion of indium after subcutaneous injection of citrated indium-114m trichloride in rats was represented by two components, one with a half-time of three days and the other with a half-time of 94 days. Two components to the excretion curve were also found after intramuscular injection of indium-114m trichloride. These components had half-times of 6.5 days and 126 days, respectively. The rate of excretion was determined by analysis of the urine and feces and was probably affected by the slow rate of absorption from the site of injection.

Castronovo and Wagner (21) examined the excretion of ^{114m}In-labeled ionic indium and hydrated indium hydroxide after intravenous injection in mice. The excretion curve was found to have two components in both cases. The fast component of the ionic indium excretion curve accounted for 31% to 52% of the dose and had a half-time of 1.9 to 2.1 days, while the slow component had a half-time of 69 to 74.5 days. The fast component of the hydrated indium hydroxide excretion had a half-time of two days and accounted for 18% to 28% of the dose whereas the slow component had a half-time of 61.9 to 73.8 days. The total body radioactivity of the mice was measured for up to 70 days after injection.

Neither Smith and co-workers (19) nor Castronovo and

Wagner (21) mentioned analysis of their data for a very slowly excreted fraction of the radioactivity. In the present investigation, when no correction was made for the very slowly excreted fraction, analysis of the data indicated that the longest-lived component had a half-life of 55.0 days. However, when the correction was made, the half-life of this component was calculated as being 18.3 days. It was felt that, unless the investigation was carried on for a very long period of time and virtually all of the radioactivity in the animals excreted, the data should be analyzed for a very slowly excreted fraction. If present, this slowly excreted fraction should be taken into consideration.

E. Toxicity Studies in Mice

1. Toxicity of Indium Oxinate Particles

To study the toxicity of the radiopharmaceutical being investigated, suspensions of various concentrations of indium oxinate particles in 2% PVP were administered to groups of mice. The acute toxicity results are shown in Table II.

Although all surviving animals were observed for at least one month, all deaths occurred within 12 hours of administration. All mice that received 125 and 150 mg of indium oxinate per kg showed immediate respiratory difficulty. The one death at 125 mg per kg and one of the deaths

TABLE II

ACUTE TOXICITY OF INDIUM OXINATE PARTICLES IN MICE AFTER INTRAVENOUS INJECTION

Dose (mg per kg)	Number of Animals	Number of Deaths
50	5	0
100	9	0
125	4	1
150	4	3

at 150 mg per kg occurred within a few minutes after injection and appeared to be due to the acute respiratory difficulty. This was probably caused by the injection of a large number of insoluble particles (20). As it was not possible to distinguish between chemical toxicity of the compound and the effect of injecting particles, the LD_{50} was not calculated.

Five of the animals, injected with 100 mg of indium oxinate per kg, were weighed at approximately weekly intervals for 40 days. The weights at each time period were compared to those of control animals. At none of the time periods was there any statistical difference between the weights of the controls and those administered indium oxinate. None of the animals showed either weight loss or below normal weight gain.

It has been reported that the lungs, liver and kidneys were affected by the administration of indium (6,20). In histopathological studies of the lung, liver and kidney tissue of mice sacrificed 24 hours, 17 days and 48 days

after intravenous injection of 100 mg indium oxinate per kg no pathological changes were observed in either the liver or kidney samples. Examination of the lungs revealed the presence of particulate matter in blood vessels, endothelial hyperplasia with infarction, and some edema. Some lung tissue fibrosis appeared in the animal sacrificed after 48 days. It is to be noted that the dose administered was in the order of 10^4 times that which would be normally administered to humans.

2. Acute Toxicity of Oxine Particles in Mice

The ^{113m}In-indium oxinate suspension, when prepared as previously described, contained a considerable proportion of oxine. Therefore, the toxicity of oxine particles, in the size range of 10 to 30 microns, was studied.

Oxine, as a suspension in 2% PVP, was administered in various concentrations to groups of mice by tail vein injection. The results are shown in Table III.

TABLE III

ACUTE TOXICITY OF OXINE PARTICLES IN MICE AFTER INTRAVENOUS ADMINISTRATION

Dose (mg per kg)	Number of Animals	Number of Deaths
40	5	1
50	5	1
75	5	1
100	5	3

The surviving animals were observed for at least three weeks after injection. All deaths occurred within 24 hours of injection. All animals that were injected with oxine in the dose ranges investigated demonstrated an immediate respiratory response. In fact, all deaths except one at the 100 mg per kg level appeared to be due to this respiratory difficulty. One animal died between 21 and 24 hours following injection, after evidence of the immediate respiratory response had already disappeared. As was the case with the indium oxinate toxicity studies, it was not possible to differentiate between the lethal effect of the particles and the chemical toxicity of oxine. Therefore, the LD_{50} was not calculated.

As previously stated, the ^{113m}In-indium oxinate suspension contains 250 µg of indium oxinate and 300 µg of oxine per ml of generator eluate. Assuming that 2 ml of generator eluate were used in a lung scan of a 70 kg human, the doses of indium oxinate and oxine would be about 7µg per kg and about 9 µg per kg, respectively. Based on the results of the toxicity studies in mice, there appears to be a safety factor of at least 10^3 for each of these chemicals when administered in particulate form.

F. Organ Scans

A concentration of radioactivity in the lungs of mice had been observed after the intravenous administration

of ^{113m}In-indium oxinate suspension. However, it was necessary to confirm that a similar localization of radioactivity would also occur in large animals.

A lung scan of a rabbit was obtained after the intravenous injection of 300 μCi of ^{113m}In-indium oxinate suspension. The scan is shown in Figure 13. The lung scan of a dog obtained after the intravenous administration of 800 μCi of ^{113m}In-indium oxinate is shown in Figure 14.

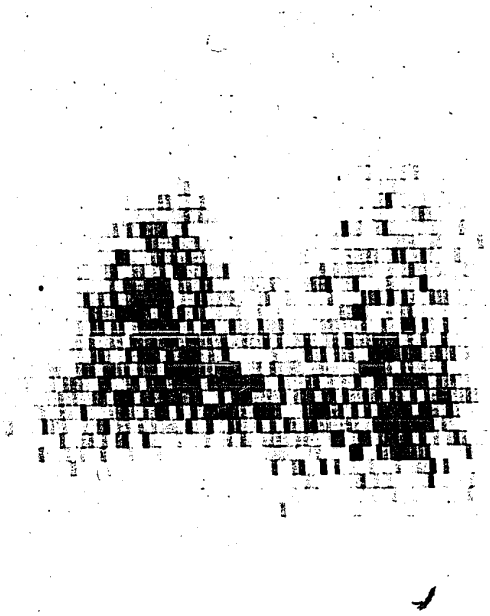
As can be noted from the scans, the radioactivity appeared to be well distributed in the lungs. Very little radioactivity appeared in the liver. From these scans, it was concluded that the ^{113m}In-indium oxinate particles were efficiently cleared from the circulatory system by the capillaries of the lungs in the rabbit and the dog.

G. Absorbed Radiation Dose Calculations for ^{113m}In-Indium Oxinate Suspension

Since ^{113m}In-indium oxinate suspension appeared to be of potential value in diagnostic medicine, estimates were made of the radiation dose delivered to the whole body and to the target organs, the lungs and the liver. These calculations were based on the following assumptions:

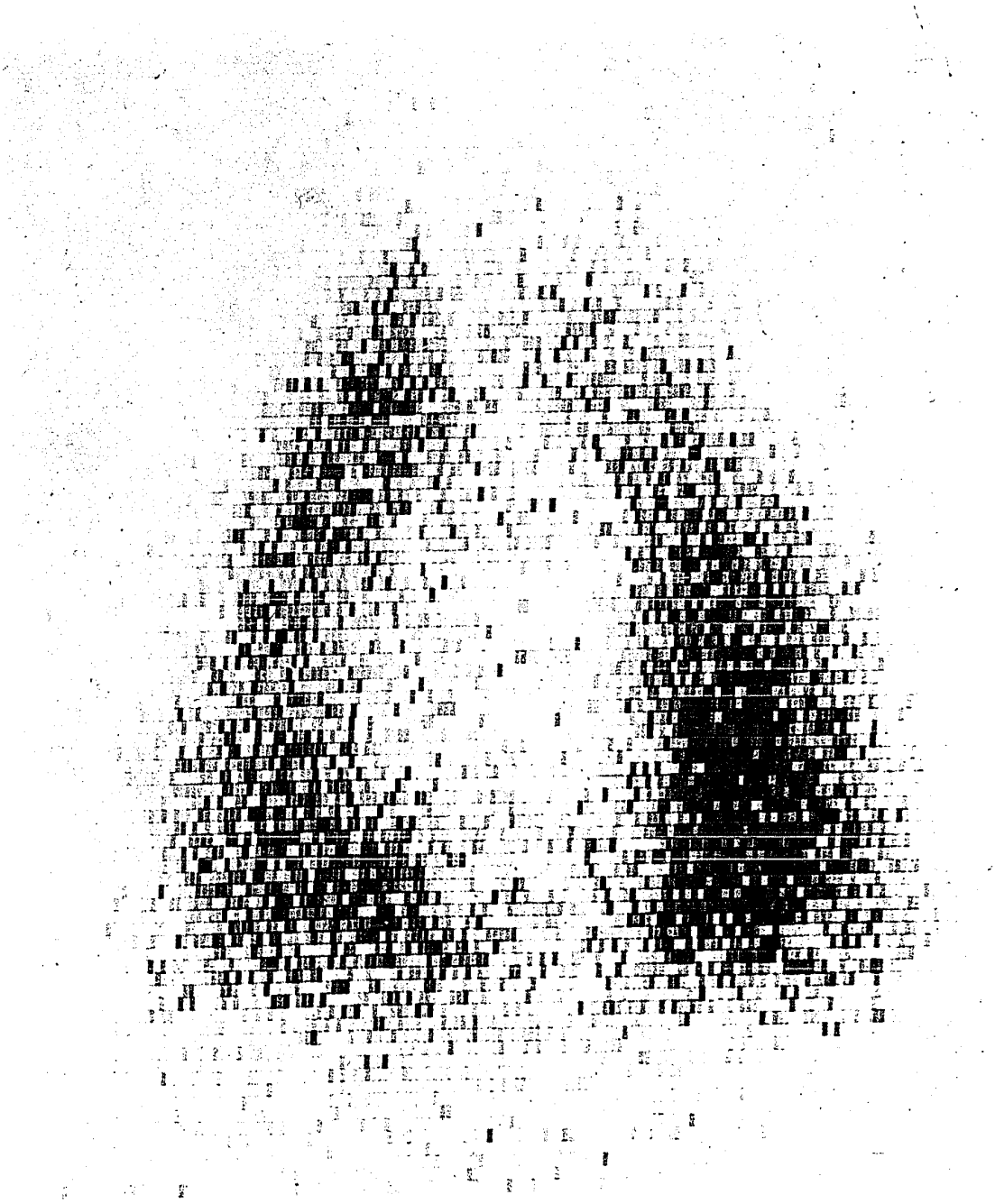
i) internal distribution and elimination found in animal studies using mice can be applied to the human body, and

ii) the human body has a lung mass of 999 g,



Lung Scan After Injection
of 300 μCi of ^{113m}In-Indium Oxinate Suspension
Into a Rabbit

Figure 13



Lung Scan After Injection of 800 μCi
of ^{113m}In-Indium Oxinate Suspension Into a Dog

Figure 14

a liver mass of 1,833 g and a total body mass of 70 kg (95).

Based on the animal distribution and elimination studies certain generalities were made:

i) the uptake half-time in the lungs was negligible;

ii) the biological elimination half-time in the liver was so large, in comparison to the half-life of indium-113m, that it could be ignored;

iii) the original concentration in the lungs was 95.2% of the dose;

iv) the biological elimination half-time from the lungs was 26.1 hours;

v) the radioactivity which is not initially deposited in the lung (4.8% of the dose) was concentrated, without appreciable uptake half-time, by the liver;

vi) the radioactivity released from the lungs was taken up by the liver with an uptake half-time equivalent to the elimination half-time of the activity from the lungs.

The last three generalities are not strictly true on the basis of the results of distribution studies. However, it is believed that these represent the extreme situation in which the radiation dosage would be the highest.

The absorbed dose calculations are based on the

equation (96):

$$\bar{D}(v \leftarrow r) = \frac{\tilde{A}_r}{M_v} \Sigma \Delta_i \phi_i (v \leftarrow r) \text{ rad}$$

where $\bar{D}(v \leftarrow r)$ = dose to volume v from a uniform distribution of radioactivity throughout region r;

Δ_i = equilibrium dose constant for radiation of type i = 1, 2, 3, --- with a fractional frequency n_i per disintegration and a mean energy $\bar{E}_i$ in MeV

$$= 2.13\ n_i\ \bar{E}_i \left[\frac{\text{g-rad}}{\mu\text{Ci-hr}}\right]$$

$\phi_i(v \leftarrow r)$ = the absorbed dose fraction in volume v

$\tilde{A}_r$ = the cumulative activity (μCi-hr)

M_v = mass of volume v under consideration

Indium-113m decays by a monoenergetic gamma emission of 393 KeV. However, approximately 35% of emissions result in internal conversion electrons (97). Tables IV and V show the values of Δ_i and ϕ_i used in these calculations.

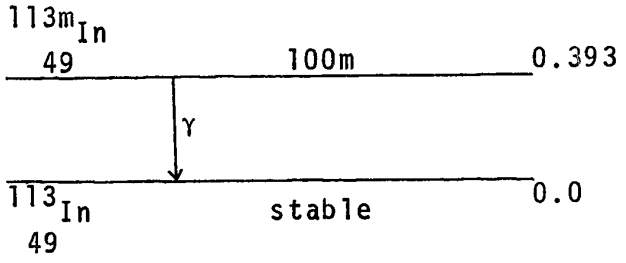


Figure 15

Decay Scheme of ^{113m}In (97)

TABLE IV

EQUILIBRIUM DOSE CONSTANTS (Δ_i) AND ABSORBED DOSE FRACTIONS (ϕ_i) FOR VARIOUS EMISSIONS OF ^{113m}In OF UNIFORM DISTRIBUTION IN THE LUNGS

Radiation (i)	n_i	$\bar{E}_i$(MeV)	$\Delta_i \left[\frac{\text{g-rad}}{\mu\text{Ci-hr}}\right]$(a)	ϕ_i Lungs(b)	ϕ_i Liver(b)	ϕ_i Whole Body(b)
Gamma 1	0.654	0.3930	0.5474	0.0514	0.0155	0.354
K Int. Con. Electron	0.281	0.3651	0.2185	1.0	0	1.0
L Int. Con. Electron	0.049	0.3891	0.0406	1.0	0	1.0
M Int. Con. Electron	0.016	0.3923	0.0136	1.0	0	1.0
K α-1 X-rays	0.131	0.0242	0.0068	0.475	0.0142	0.981
K α-2 X-rays	0.066	0.0240	0.0034	0.475	0.0142	0.981
K β-1 X-rays	0.034	0.0273	0.0020	0.231	0.0258	0.878
K β-2 X-rays	0.007	0.0279	0.0004	0.231	0.0258	0.878
L X-rays	0.034	0.0033	0.0002	0.815	0.0003	0.996
KLL Auger Electron	0.029	0.0201	0.0012	1.0	0	1.0
KLX Auger Electron	0.012	0.0234	0.0006	1.0	0	1.0
KXY Auger Electron	0.002	0.0266	0.0001	1.0	0	1.0
LMM Auger Electron	0.282	0.0026	0.0016	1.0	0	1.0
MXY Auger Electron	0.664	0.0007	0.0010	1.0	0	1.0
				$\Sigma\Delta_i\phi_i$ = 0.3109	$\Sigma\Delta_i\phi_i$ = 0.0088	$\Sigma\Delta_i\phi_i$ = 0.4834

(a) from Dillman (97); (b) from Snyder et al. (95)

TABLE V

EQUILIBRIUM DOSE CONSTANT (Δ_i) AND ABSORBED DOSE FRACTIONS (ϕ_i)
FOR VARIOUS EMISSIONS OF ^{113m}In OF UNIFORM DISTRIBUTION IN THE LIVER

Radiation (i)	n_i	$\bar{E}_i$(MeV)	$\Delta_i \left[\frac{\text{g-rad}}{\mu\text{Ci-hr}}\right]$(a)	ϕ_i Lungs(b)	ϕ_i Liver(b)	ϕ_i Whole Body(b)
Gamma 1	0.654	0.3930	0.5474	0.0084	0.157	0.407
K Int. Con. Electron	0.281	0.3651	0.2185	0	1.0	1.0
L Int. Con. Electron	0.049	0.3891	0.0406	0	1.0	1.0
M Int. Con. Electron	0.016	0.3923	0.0136	0	1.0	1.0
K α-1 X-rays	0.131	0.0242	0.0068	0.0086	0.784	0.984
K α-2 X-rays	0.066	0.0240	0.0034	0.0086	0.784	0.984
K β-1 X-rays	0.034	0.0273	0.0020	0.0165	0.543	0.905
K β-2 X-rays	0.007	0.0279	0.0004	0.0165	0.543	0.905
L X-rays	0.034	0.0033	0.0002	0.0001	0.967	0.996
KLL Auger Electron	0.029	0.0201	0.0012	0	1.0	1.0
KLX Auger Electron	0.012	0.0234	0.0006	0	1.0	1.0
KXY Auger Electron	0.002	0.0266	0.0001	0	1.0	1.0
LMM Auger Electron	0.282	0.0026	0.0016	0	1.0	1.0
MXY Auger Electron	0.664	0.0007	0.0010	0	1.0	1.0
				$\Sigma\Delta_i\phi_i$ = 0.0049	$\Sigma\Delta_i\phi_i$ = 0.3727	$\Sigma\Delta_i\phi_i$ = 0.5124

(a) from Dillman (97); (b) from Snyder et al. (95)

1. Radiation Dose to the Lungs

Based on the assumptions previously stated, the radiation dose delivered to the lungs is composed of the dose from the radioactivity distributed in the lungs and the dose from the radioactivity distributed in the liver. The radioactivity in the lungs was initially 95.2% of the administered dose and was cleared from the lungs with a biological half-time of 26.1 hours. The radioactivity in the liver consisted of 4.8% of the administered dose initially distributed in the liver and 95.2% of the administered dose which had an uptake half-time in the liver of 26.1 hours.

Indium-113m has a physical half-life of 1.67 hours. Therefore,

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}} = \frac{1}{1.67\ hr} + \frac{1}{26.1\ hr}$$

$$\frac{1}{T_{eff}} = 0.6371\ hr^{-1}$$

where T_{eff} is the effective half-time of ^{113m}In in the lung.

$$\lambda_{eff} = 0.693 \times \frac{1}{T_{eff}}$$

$$= 0.4415\ hr^{-1}$$

$\tilde{A}_r = A_r\left[\frac{1}{\lambda_{eff}}\right]$ when there is only one component to the biological clearance (96).

It was found that 95.2% of the dose concentrates initially in the lungs; therefore, for each mCi adminis-

tered, 952 μCi will go to the lungs.

$$\bar{D}(v \leftarrow r) = \frac{A_r}{M_v}\left[\frac{1}{\lambda_{eff}}\right] \Sigma \Delta_i \phi_i (v \leftarrow r)$$

Therefore,

$$\bar{D}(v \leftarrow r) = \frac{952\ \mu Ci}{999\ g} \times \frac{1}{0.4415\ hr^{-1}} \times 0.3109\ \frac{g\text{-}rad}{\mu Ci\text{-}hr}$$

$$= 0.6711\ rad/mCi$$

For each millicurie of radioactivity administered 48 μCi (4.8%) was initially distributed in the liver. Since the biological half-time for clearance from the liver was so large, it can be ignored. Therefore, $T_{eff} = T_{phys}$.

$$\lambda_{eff} = \frac{0.693}{T_{phys}}$$

$$= 0.4150\ hr^{-1}$$

The dose to the lungs from the activity originally in the liver was:

$$\bar{D}(v \leftarrow r) = \frac{A_r}{M_v}\left[\frac{1}{\lambda_{eff}}\right] \Sigma \Delta_i \phi_i (v \leftarrow r)$$

$$= \frac{48\ \mu Ci}{999\ g} \times \frac{1}{0.4150\ hr^{-1}} \times 0.0049\ \frac{g\text{-}rad}{\mu Ci\text{-}hr}$$

$$= 5.67 \times 10^{-4}\ rad/mCi$$

Assuming that all radioactivity that was released from the lung was taken up by the liver, then the uptake half-time of this radioactivity by the liver was 26.1 hours. The cumulative activity $\tilde{A}$ in this case was calculated using

the method of Smith (98):

$$\bar{A} = A_0 \left[\frac{\lambda_{eff-u} - \lambda_{eff-e}}{(\lambda_{eff-u})(\lambda_{eff-e})} \right] \mu Ci\text{-hr}$$

where λ_{eff-u} = effective uptake constant

$= \lambda_u + \lambda$

λ_{eff-e} = effective elimination constant

$= \lambda_e + \lambda$

where λ_u is the biological uptake constant

λ_e is the biological elimination constant

λ is the physical decay constant

$$\lambda = \frac{0.693}{1.67} = 0.4150 \text{ hr}^{-1}$$

$$\lambda_u = \frac{0.693}{26.1} = 0.0266 \text{ hr}^{-1}$$

Therefore,

$$\lambda_{eff-u} = 0.1450 + 0.0266$$

$$= 0.4416 \text{ hr}^{-1}$$

Since λ_e is negligible, $\lambda_{eff-e} = \lambda = 0.4150 \text{ hr}^{-1}$

$$\bar{A}_r = A_r \left[\frac{0.4416 - 0.4150}{(0.4416)(0.4150)} \right]$$

$$= 0.1451 \, A_r \ \mu Ci\text{-hr}$$

For an administered dose of 1mCi, A_r = 952 μCi

$$\bar{D}(w \leftarrow r) = \frac{(0.1451)(952) \ \mu Ci\text{-hr}}{999g} \times 0.0049 \ \frac{g\text{-rad}}{\mu Ci\text{-hr}}$$

$$= 6.77 \times 10^{-4} \text{ rad/mCi}$$

The total radiation dose to the lungs was estimated to be 0.6711 rad + 0.000567 rad + 0.000677 rad = 0.6723 rad per millicurie of administered radioactivity.

2. Radiation Dose to the Liver

Detailed calculations of the radiation dose to the liver are shown in Appendix 2. The total radiation dose to the liver was estimated to be 0.0620 rad per millicurie of radioactivity administered.

3. Radiation Dose to the Whole Body

The radiation dose to the whole body was estimated to be 0.0168 rad per millicurie of indium-113 administered as indium oxinate particles. Detailed calculations are given in Appendix 2.

Thus, based on the results of tissue distribution studies in mice, the radiation dosage was estimated to be 0.67 rad per mCi to the lungs, 0.06 rad per mCi to the liver and 0.02 rad per mCi to the whole body of the human model. Published estimates of radiation dosages of currently used lung scanning agents to the lungs include 0.75 rad and 0.55 rad per mCi for ^{113m}In-ferric hydroxide (6,99), and 0.28 rad and 0.62 rad per mCi for ^{99m}Tc-ferric hydroxide (100,82). Smith et al. (101) have estimated the radiation dosage from 300 μCi of ^{131}I-macroaggregated albumin to be 1.9 rads to the lungs, 0.40 rad to the liver and 0.1 rad to the whole body.

SUMMARY AND CONCLUSIONS

1) After the intravenous administration into mice of ^{113m}In-indium oxinate as an ethanolic solution, the radioactivity was cleared from the blood at a relatively slow rate. No other tissue showed an ability to concentrate the radioactivity to an appreciable extent.

2) When an ethanolic solution of ^{114m}In-indium oxinate that was near saturation was administered intravenously into mice approximately 75% of the radioactivity was concentrated in the lungs within fifteen minutes.

3) A method of relatively rapid preparation of an indium oxinate suspension was developed. When prepared by this method, a ^{113m}In-indium oxinate suspension contained approximately 750,000 particles in the size range 10 to 30 microns for each milliliter of generator eluate used.

4) Ten minutes after intravenous injection of ^{114m}In-indium oxinate suspension into mice, greater than 90% of the radioactivity was concentrated in the lungs. As the concentration in the lungs decreased, there was an increase in the levels of radioactivity in other tissues, particularly the liver, spleen, muscle and kidneys, which reached a maximum three to seven days after administration.

5) Analysis of the whole body burden of radioactivity for 35 days after the intravenous administration into mice of ^{114m}In-indium oxinate indicated that excretion could be resolved into three exponential components and a large bound or very slowly excreted portion. The three components that were resolved had half-times of 1.3 ± 0.1 days, 4.1 ± 0.1 days and 18.3 ± 0.3 days and represented 19.7%, 6.6% and 39.8% of the administered radioactivity, respectively. The very slowly excreted portion of the radioactivity appeared to consist of 38.6% of the administered dose.

6) Attempts at extraction of the excreted radioactivity from the urine and feces with chloroform were unsuccessful, indicating that the indium is not excreted as the oxinate. However, due to deficiencies in the experimental design, no conclusions can be drawn from these results.

7) Indium oxinate particles in suspension, when administered intravenously at a dose of 100 mg per kg, produced no observable gross effects in mice. Histopathological examination of the liver and kidneys revealed no changes. However, the lungs had some pathological changes. Endothelial hyperplasia with infarction, edema, and fibrosis was noted.

8) Oxine particles in suspension caused respiratory difficulties when administered at dosage levels as low as 40 mg per kg.

9) The preparation of ^{113m}In-indium oxinate suspension was reproducible. Greater than 90% of the radioactivity was concentrated in the lungs after intravenous administration of the suspension into mice. Lung scans of a rabbit and a dog indicated that the majority of the radioactivity was concentrated in the lungs after the intravenous injection of ^{113m}In-indium oxinate.

10) The radiation dose to a human model, based on tissue distribution studies in mice, was estimated to be 0.67 rad to the lungs, 0.06 rad to the liver and 0.02 rad to the whole body for each millicurie of ^{113m}In-indium oxinate.

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APPENDIX 1

TABLE 1

TISSUE DISTRIBUTION IN MICE AFTER INTRAVENOUS ADMINISTRATION OF 30 μCi OF ^{113m}In-INDIUM OXINATE SOLUTION[a,b]

Tissue	Time After Administration		
	1 Hour	6 Hours	12 Hours
Blood[c]	47.76 ± 5.45	19.33 ± 4.50	22.29 ± 3.25
Heart	2.00 ± 1.13	0.70 ± 0.31	1.08 ± 0.36
Lungs	13.55 ± 3.69	8.52 ± 1.50	7.45 ± 1.46
Liver	7.00 ± 0.08	5.44 ± 2.30	8.39 ± 2.39
Kidneys	5.57 ± 0.28	6.77 ± 2.44	8.56 ± 2.86
Spleen	0.75 ± 0.08	1.81 ± 1.82	1.82 ± 0.99
Brain	0.68 ± 0.35	0.14 ± 0.02	0.15 ± 0.07
Muscle[d]	0.94 ± 0.08	0.60 ± 0.13	0.86 ± 0.24
Fat[d]	0.69 ± 0.35	0.33 ± 0.08	0.71 ± 0.18

[a] Expressed as mean of three animals ± standard deviation

[b] Percent of injected activity in whole tissue except as indicated

[c] Percent of injected activity per milliliter of blood

[d] Percent of injected activity per gram of tissue

TABLE 2

TISSUE DISTRIBUTION IN MICE AFTER INTRAVENOUS ADMINISTRATION OF 4 μCi OF ^{114m}In-INDIUM OXINATE SOLUTION[a,b]

Tissue	Time After Administration			
	15 Minutes	6 Hours	24 Hours	72 Hours
Blood[c]	14.84 ± 1.05	14.76 ± 3.28	10.73 ± 5.61	7.18 ± 1.24
Heart	0.32 ± 0.18	1.22 ± 0.46	1.51 ± 1.11	0.35 ± 0.01
Lungs	74.88 ± 1.48	41.90 ± 2.23	20.02 ± 13.94	15.69 ± 6.31
Liver	3.88 ± 0.15	5.62 ± 0.37	5.53 ± 0.55	8.45 ± 1.67
Kidneys	1.41 ± 0.06	2.48 ± 0.15	3.68 ± 1.20	5.55 ± 0.64
Brain	0.04 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.08 ± 0.05
Muscle[d]	0.43 ± 0.16	0.57 ± 0.05	0.96 ± 0.26	0.44 ± 0.40

[a] Expressed as mean of three animals ± standard deviation

[b] Percent of injected activity in whole tissue except as indicated

[c] Percent of injected activity per milliliter of blood

[d] Percent of injected activity per gram of tissue

TABLE 3

TISSUE DISTRIBUTION IN MICE AFTER INTRAVENOUS ADMINISTRATION OF 5 μCi OF ^{114m}In-INDIUM OXINATE SUSPENSION[a,b]

Tissue	Time After Administration				
	10 Minutes	3 Hours	6 Hours	12 Hours	18 Hours
Blood[c]	0.08 ± 0.01	0.07 ± 0.01	0.19 ± 0.03	0.53 ± 0.17	0.72 ± 0.11
Heart	0.10 ± 0.02	0.10 ± 0.02	0.41 ± 0.13	0.35 ± 0.12	0.41 ± 0.10
Lungs	95.20 ± 4.71	73.85 ± 2.60	69.13 ± 6.66	56.76 ± 3.98	49.30 ± 5.87
Liver	2.62 ± 0.40	5.85 ± 0.31	18.26 ± 1.88	20.71 ± 3.80	22.61 ± 3.24
Kidneys	0.27 ± 0.05	0.39 ± 0.07	1.09 ± 0.14	1.25 ± 0.28	1.60 ± 0.55
Spleen	0.16 ± 0.03	0.51 ± 0.06	1.78 ± 0.18	2.23 ± 0.21	2.05 ± 0.48
Brain	0.06 ± 0.02	0.06 ± 0.01	0.21 ± 0.03	0.20 ± 0.06	0.19 ± 0.06
Muscle[d]	0.13 ± 0.02	0.20 ± 0.06	0.63 ± 0.15	0.78 ± 0.34	0.65 ± 0.13
Fat[d]	0.05 ± 0.05	0.06 ± 0.04	0.16 ± 0.04	0.30 ± 0.14	0.29 ± 0.05

... continued

TABLE 3 (continued)

Tissue	Time After Administration				
	24 Hours	3 Days	7 Days	14 Days	21 Days
Blood[c]	0.73 ± 0.24	1.16 ± 0.13	0.61 ± 0.06	0.23 ± 0.05	0.17 ± 0.01
Heart	0.44 ± 0.13	0.63 ± 0.11	0.54 ± 0.10	0.33 ± 0.06	0.30 ± 0.06
Lungs	42.58 ± 4.93	11.92 ± 2.09	4.75 ± 1.17	2.56 ± 1.07	2.43 ± 0.34
Liver	22.26 ± 8.34	31.20 ± 2.65	30.02 ± 7.72	22.57 ± 2.62	17.04 ± 3.05
Kidneys	1.94 ± 0.46	3.57 ± 0.51	3.44 ± 1.32	1.53 ± 0.23	1.18 ± 0.13
Spleen	2.18 ± 0.78	2.60 ± 0.53	3.76 ± 0.85	2.53 ± 0.21	1.60 ± 0.38
Brain	0.14 ± 0.05	0.09 ± 0.02	0.12 ± 0.02	0.10 ± 0.03	0.07 ± 0.02
Muscle[d]	0.75 ± 0.24	1.28 ± 0.35	2.53 ± 0.48	1.93 ± 0.40	1.50 ± 0.22
Fat[d]	0.36 ± 0.07	0.44 ± 0.13	0.41 ± 0.02	0.43 ± 0.09	0.24 ± 0.03

[a] Expressed as mean of five animals ± standard deviation

[b] Percent of injected activity in whole tissue except as indicated

[c] Percent of injected activity per milliliter of blood

[d] Percent of injected activity per gram of tissue

TABLE 4

BODY BURDEN OF ^{114m}In IN MICE FOLLOWING INTRAVENOUS INJECTION OF 5 μCi OF ^{114m}In-INDIUM OXINATE SUSPENSION[a]

Time After Injection (Days)	Percent Of Dose Remaining[b]	Time After Injection (Days)	Percent Of Dose Remaining[b]
0	100	18	59.9 ± 3.2
1	100.3 ± 5.8	19	59.4 ± 3.7
2	98.4 ± 4.6	20	58.8 ± 3.8
3	93.8 ± 3.6	21	58.6 ± 4.0
4	87.2 ± 2.2	22	57.5 ± 3.6
5	82.0 ± 3.4	23	56.6 ± 3.3
6	77.8 ± 3.7	24	55.7 ± 3.4
7	74.4 ± 2.7	25	55.5 ± 3.4
8	72.7 ± 3.5	26	54.5 ± 3.6
9	71.1 ± 3.8	27	53.8 ± 3.2
10	68.3 ± 3.8	28	52.9 ± 2.9
11	66.8 ± 3.5	29	52.6 ± 3.0
12	66.0 ± 3.9	30	52.2 ± 3.6
13	64.4 ± 2.9	31	52.2 ± 3.5
14	63.6 ± 3.9	32	51.6 ± 3.2
15	63.2 ± 3.9	33	50.6 ± 3.7
16	62.2 ± 3.5	34	50.7 ± 3.3
17	61.2 ± 3.4	35	49.9 ± 2.9

[a] Determined by whole body counting

[b] Mean of five animals ± standard deviation

APPENDIX 2

CALCULATIONS OF THE ABSORBED RADIATION DOSE TO THE LIVER AND WHOLE BODY FROM ^{113m}In-INDIUM OXINATE SUSPENSION

A. Absorbed Radiation Dose to the Liver

1. Radiation dose to liver from radioactivity in lungs

For 1 mCi administered there were 952 μCi originally in the lungs:

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}} = \frac{1}{1.67 \text{ hr.}} + \frac{1}{26.1 \text{ hr.}}$$

$$= 0.6371 \text{ hr}^{-1}$$

$$\lambda_{eff} = 0.693 \times \frac{1}{T_{eff}}$$

$$= 0.4415 \text{ hr}^{-1}$$

$$\bar{D}(v \leftarrow r) = \frac{A_r}{M_v}\left[\frac{1}{\lambda_{eff}}\right]\Sigma\Delta_i\phi_i(v \leftarrow r) \text{ rad}$$

$$= \left[\frac{952 \text{ μCi}}{1833 \text{ g}}\right]\left[\frac{1}{0.4415 \text{ hr}^{-1}}\right]\left[0.0088 \frac{\text{g-rad}}{\text{μCi-hr}}\right]$$

$$= 0.0104 \text{ rad per mCi}$$

2. Radiation dose to liver from radioactivity in liver:

a) Dose to liver from radioactivity initially in liver:

For 1 mCi administered there were originally 48 μCi distributed in the liver.

$$\lambda_{eff} = \frac{0.693}{T_{phys}}$$

$$= 0.4150 \text{ hr}^{-1}$$

$$\bar{D}(v \leftarrow r) = \frac{A_r}{M_v}\left[\frac{1}{\lambda_{eff}}\right]\Sigma\Delta_i\phi_i(v \leftarrow r) \text{ rad}$$

$$= \left[\frac{48\ \mu Ci}{1833\ g}\right]\left[\frac{1}{0.4150\ hr^{-1}}\right]\left[0.3727\ \frac{g\text{-rad}}{\mu Ci\text{-hr}}\right]$$

$$= 0.0235 \text{ rad per mCi}$$

b) Dose to liver from radioactivity released by lungs and taken up by liver.

$$\lambda = \frac{0.693}{1.67\ hr} = 0.4150\ hr^{-1}$$

$$\lambda_u = \frac{0.693}{26.1\ hr} = 0.0266\ hr^{-1}$$

$$\lambda_{eff\text{-}u} = 0.4150 + 0.0266 = 0.4416\ hr^{-1}$$

Since λ_e is negligible,

$$\lambda_{eff\text{-}e} = \lambda = 0.4150\ hr^{-1}$$

$$\tilde{A} = A_r\left[\frac{\lambda_{eff\text{-}u} - \lambda_{eff\text{-}e}}{(\lambda_{eff\text{-}u})\ (\lambda_{eff\text{-}e})}\right]\mu Ci\text{-hr}$$

$$= A_r\left[\frac{0.4416 - 0.4150}{(0.4416)\ (0.4150)}\right]$$

$$= 0.1451\ A_r\ \mu Ci\text{-hr}$$

$$\bar{D}(v \leftarrow r) = \frac{0.1451\ A_r}{M_v}\Sigma\Delta_i\phi_i(v \leftarrow r) \text{ rad}$$

$$= \left[\frac{(0.4151)(952)\ \mu Ci\text{-hr}}{1833\ g}\right]\left[0.3727\ \frac{g\text{-rad}}{\mu Ci\text{-hr}}\right]$$

$$= 0.0281 \text{ rad per mCi}$$

Therefore the total absorbed dose to the liver is calculated to be 0.0104 rad + 0.0235 rad + 0.0281 rad = 0.0620 rad per mCi.

B. Absorbed Dose to the Whole Body

1. Radiation dose to whole body from radioactivity in the lungs

$$\lambda_{eff} = 0.4415 \text{ hr}^{-1}$$

$$\overline{D}(v \leftarrow r) = \frac{A_r}{Mv}\left[\frac{1}{\lambda_{eff}}\right]\Sigma\Delta_i\phi_i(v \leftarrow r) \text{ rad}$$

$$= \left[\frac{952 \text{ } \mu\text{Ci}}{70{,}000 \text{ g}}\right]\left[\frac{1}{0.4415 \text{ hr}^{-1}}\right]\left[0.4834 \frac{\text{g-rad}}{\mu\text{Ci-hr}}\right]$$

$$= 0.0149 \text{ rad per mCi}$$

2. Radiation dose to whole body from radioactivity in liver

a) dose to whole body from radioactivity initially in liver

$$\lambda_{eff} = 0.4150 \text{ hr}^{-1}$$

$$\overline{D}(v \leftarrow r) = \frac{A_r}{M_v}\left[\frac{1}{\mu_{eff}}\right]\Sigma\Delta_i\phi_i(v \leftarrow r) \text{ rad}$$

$$= \left[\frac{48 \text{ } \mu\text{Ci}}{70{,}000 \text{ g}}\right]\left[\frac{1}{0.4150 \text{ hr}^{-1}}\right]\left[0.5124 \frac{\text{g-rad}}{\mu\text{Ci-hr}}\right]$$

$$= 0.0009 \text{ rad per mCi}$$

b) Dose to whole body from radioactivity released by lungs and taken up by liver

$$\tilde{A} = 0.1451\ A_r\ \mu Ci\text{-hr}$$

$$\overline{D}(v \leftarrow r) = \frac{0.1451 A_r}{M_v} \Sigma \Delta_i \phi_i (v \leftarrow r)\ rad$$

$$= \left[\frac{(0.1451)(952)\ \mu Ci\text{-hr}}{70{,}000\ g}\right] \left[0.5124\ \frac{g\text{-rad}}{\mu Ci\text{-hr}}\right]$$

$$= 0.0010\ rad\ per\ mCi$$

Therefore the total dose to the whole body is estimated to be 0.0149 rad + 0.0009 rad + 0.0010 rad = 0.0168 rad per mCi.

END OF REEL