COMPARATIVE TOXICITY AND PHARMACODYNAMICS OF IONIC INDIUM CHLORIDE AND HYDRATED INDIUM OXIDE

Frank P. Castronovo, Jr. and Henry N. Wagner, Jr.

The Johns Hopkins Medical Institutions, Baltimore, Maryland

Interest in the toxicity and pharmacodynamics of indium compounds resulted from their use as diagnostic radiopharmaceuticals. Indium-113 ($T_{1/2}$ 1.7hr) is administered intravenously as ionic indium chloride, colloidal hydrated indium oxide, and macroaggregated ^{113}In-iron hydroxide. Indium-111 ($T_{1/2}$ 2.8 days) is also used in clinical nuclear medicine. Studies included determination of the (LD_{50}/4 days) of ionic and colloidal indium and a pharmacodynamic evaluation of tracer dose levels of these compounds using ^{114m}In ($T_{1/2}$ 50 days). **Initial biological** ***distribution, subsequent translocation, whole-body retention, and excretion were investigated. Colloidal hydrated indium oxide was found to be 40 times more toxic than ionic indium chloride, the principal damage being to the organs of the reticuloendothelial system. Ionic indium chloride caused acute tubular necrosis of the kidneys. Hydrated indium oxide accumulated primarily in the liver, spleen, and bone marrow and was mainly excreted in the feces. Ionic indium became protein bound after administration with subsequent translocation to the kidney and liver and was excreted mainly in the urine.***

Our interest in the toxicity and pharmacodynamics of indium resulted from its use as a diagnostic radiopharmaceutical (*1–3*). Indium is a Group III-B heavy metal widely distributed in minute quantities in nature (*4,5*). Of the 24 known isotopes of indium only two are found in nature: ^{113}In which is stable and comprises 4.33% of naturally occurring indium and ^{115}In which is radioactive with a physical half-life of 6×10^5 years (95.67% abundance) (*6*).

A number of studies on the toxicity of indium have been made before the present investigations (*7–12*): all found parenterally administered indium to be one of the most toxic of elements (*7*).

Carrier-free ^{113m}In is administered intravenously in clinical medicine in three chemical forms; ionic indium chloride at pH 3.0, colloidal hydrated indium oxide at pH 7.2, and ^{113m}In-iron hydroxide macroaggregates. Recently, ^{111}In ($T_{1/2}$ 2.8 days) has also been used in clinical nuclear medicine (*13*). When injected at pH 3.0, ionic indium is bound by the metal-binding protein transferrin (*14*). In aqueous solution at slightly acid pH or higher, ionic indium forms insoluble hydrated indium oxide (*15*).

In the present study the acute toxicity and tracer pharmacodynamics of ionic indium and colloidal hydrated indium oxide (RES agent) were compared.

MATERIALS AND METHODS

Preparation of indium compounds. The nuclides of indium used in this study were radioactive ^{114m}In (physical half-life of 50 days) and reagent grade ^{115}In (physical half-life of 6×10^5 years) which served as carrier. The ^{114m}In was obtained from Oak Ridge National Laboratory (>5 Ci/gm In), and ^{115}In was obtained from K and K Laboratories as anhydrous indium trichloride. A solution of ^{115}In containing 10 mg of trivalent indium per milliter served as a stock solution. The highest level of radioactivity administered to each animal did not exceed 10 μCi ^{114m}In.

The ionic indium chloride was prepared using a technique similar to that of Stern and Goodwin (*2*), and the colloidal hydrated indium oxide (RES agent) was prepared by using a technique similar to that of

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For reprints contact: F. P. Castronovo, Dept. of Radiology, Massachusetts General Hospital, Boston, Mass. 02114.

Goodwin, Stern, and Wagner (*3*). Both of these modified procedures have been reported in the literature (*17*).

Measurement and radioactivity. Radioactivity of all samples was measured in a Packard Series 410A well scintillation counter. Sufficient counting time was used to keep statistical errors below 5%.

Lethality. All compounds were injected into tail veins of adult male (20–25 gm) HRA/IRC white mice (purchased from Hazelton Animal Farm, Burtonsville, Md.) at the rate of 0.02 ml/sec. The levels administered were expressed as the number of milligrams of indium per kilogram body weight.

Four groups of ten mice each were used for the acute lethality (LD_{50}) studies. Each animal received a single dose (^{115}In only) of ionic indium or hydrated indium oxide. The LD_{50} at 4 days was calculated according to the method of Miller and Tainter (*16*). If deaths did not occur by 4 days, the animals survived at least 70 days when the observations were terminated.

Plasma protein binding. Two mice were injected with tracer (^{114m}In) ionic indium chloride. After 30 min the mice were killed by decapitation. The cervical venous blood from each group was collected in heparinized tubes, and the plasma was isolated after centrifugation. Two 5 λ samples were electrophoresed on starch gel.

Tissue distribution. To determine the pharmacodynamic behavior of tracer amounts of ionic and colloidal indium, studies were carried out in two groups of 36 mice for each compound. The individual groups were injected with a tracer dose of ionic or colloidal ^{114m}In (no ^{115}In added). At each of the following times after administration three mice were killed: 1, 5, 10, 15, 30 min; 1, 3, 6 hr; 1, 2, 3, and 4 days. At the time of death the liver, spleen, lung, kidney, blood (7% body weight) femur, brain, adrenal, and thyroid were isolated and their content of radioactivity measured. The percentage dose per total organ, the percentage dose per gram of tissue, and the mean ± standard deviations of each were calculated.

Whole-body retention and excretion. To study the rate of elimination of each agent from the body, two groups of ten mice each were used for whole-body retention studies. The mice were injected with tracer (^{114m}In) dose levels of either ionic or colloidal indium, and their total-body radioactivity was measured for up to 70 days after injection. For the excretion studies, three mice were injected with tracer dose levels of ionic indium; three others were injected with tracer dose levels of hydrated indium oxide. After injection each mouse was placed in a metabolic cage, and urine and feces were collected at various time intervals up to 30 days after injection. The radioactivity of urine and feces was measured in a gamma scintillation counter.

Autoradiography. A modification of Ullberg's technique (*18*) was used for the whole-body autoradiography studies. Adult male mice were injected with tracer (^{114m}In) dose levels of ionic and colloidal indium. Twenty-four hours postadministration the animals were killed by an ether overdose and immediately frozen by immersion in an acetone-dry ice mixture. The carcasses were cut into 40-μm-thick sections and contacted with a high-speed industrial film. Images were processed after 24 hr exposure time.

Organ sections of kidney, liver, and spleen were also prepared and autoradiographs obtained as described for the whole-body studies.

RESULTS

Lethality: ionic indium. The dose levels and their respective mortalities are shown in Table 1. No deaths were observed after 4 days. The lethal dose (LD_{50}) 4 days after intravenous injection was calculated to be 12.5 mg ± 0.58 (1 s.d.)/kg for the ionic indium. The slope of the probit line was

TABLE 1. LD_{50}/4 DAY STUDY

	Amount of indium ion administered (mg/kg)			
Group No.	Ionic indium	No. dead	Hydrated indium oxide	No. dead
1	7.5	0/10	0.103	0/10
2	9.8	2/10	0.207	3/10
3	12.6	4/10	0.413	7/10
4	16.5	10/10	0.825	10/10
Slope of probit line	14.82 ± 2.76		5.61 ± 1.45	
LD_{50}	12.5 mg ± 0.58/kg		0.323 mg ± 0.063/kg	

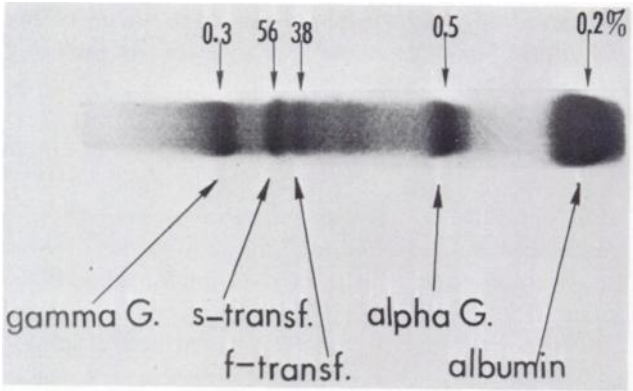

FIG. 1. Starch gel electrophoresis of plasma after i.v. injection of ^{114m}In-ionic indium. Percentage of dose in plasma is illustrated for each protein bond.

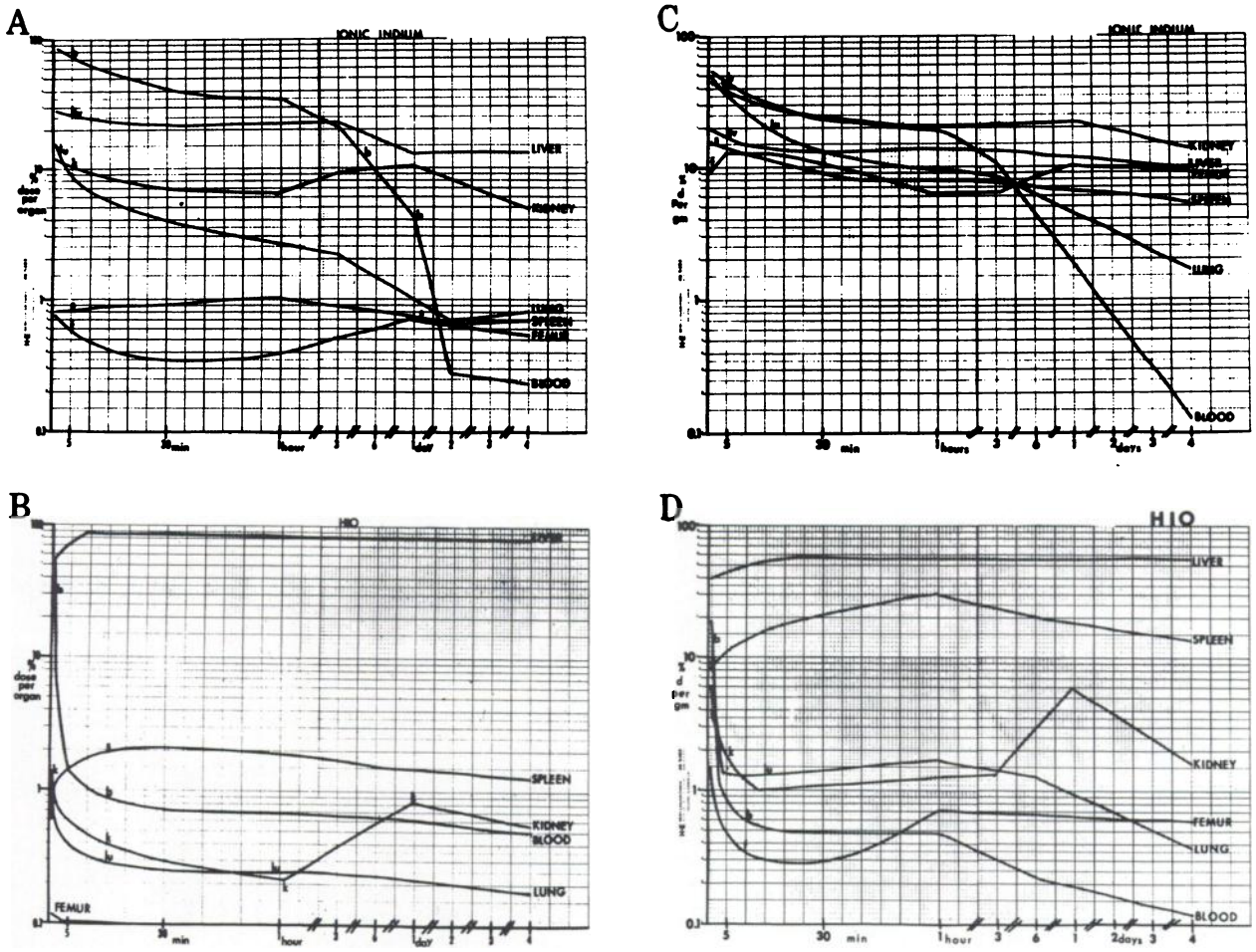

FIG. 2. Pharmacodynamic study of tracer ionic indium chloride and colloidal hydrated indium oxide in mice. (A) Ionic indium, % dose/organ. (B) Hydrated indium oxide, % dose/organ. (C) Ionic indium, % dose/gm. (D) Hydrated indium oxide, % dose/gm.

14.82 ± 2.76. Lethal amounts of ionic indium had the following effects: inactivity of the animals and hind-leg paralysis 1 day after injection; rapid and spasmodic respiration, weight loss, and abnormal pelt after 2 days; at 3 days the mice became totally inactive and died by Day 4.

Lethality: hydrated indium oxide. The dose levels of hydrated indium oxide administered along with their respective mortality rates are listed in Table 1. The lethality (LD_{50}) 4 days after injection was calculated to be 0.323 ± 0.063 mg of indium/kg. The resulting slope of the probit line was 5.16 ± 1.45. After administration of lethal amounts of hydrated indium oxide the mice lost weight, became inactive, and developed hind-leg paralysis after 2 days. They then developed muscle tremors and had clonic convulsion. Before death on Day 3, the mice bled from the mouth, nose, ears, and intestine.

Plasma protein binding of ionic indium. The results of starch gel electrophoresis are illustrated in Fig. 1. The individual protein bands consist of gamma, beta, and alpha globulins and albumin. The mouse has two transferrins (*19*) on the basis of their different electrophoretic mobilities: fast transferrin (f-trans.) and slow-transferrin (s-trans.). The tracer dose level of ionic indium became bound to both transferrins with small amounts being detected bound to gamma and alpha globulin and albumin.

Pharmacodynamics. The initial tissue distribution and subsequent translocation data for ionic indium is illustrated in Figs. 2A and C. The data are shown as the log of the percentage dose per organ or per gram as a function of time. Each time point represents the mean value for three determinations.

After intravenous injection, the blood level of tracer ionic indium fell until less than 1% of the administered dose remained in this compartment after 3 days. Concomitant with its clearance from the blood, the activity in the kidneys and liver increased showing a maximum value at 1 day. On a per gram basis, the kidneys were the principal site of uptake of ionic indium.

The initial tissue distribution and subsequent translocation data for hydrated indium oxide is illustrated in Figs. 2B and D. Almost immediately after the injection, tracer hydrated indium oxide was cleared rapidly from the blood and accumulated in the liver and spleen. Other organs which concentrated significant levels were the kidneys and bone marrow. On a per gram basis the liver accumulated the highest amount.

Whole-body retention and excretion. An initial fast component and a second slower component was observed for both compounds. The fast components accounted for about 30% of the dose for the ionic indium and 18% for the hydrated indium oxide. The biological half-life of the fast component ranged from 1.9 to 2.0 days for both compounds. The biological half-life of the slower component was similar for the ionic and particulate indium. This slower component for ionic indium was 69.0 days and for hydrated indium oxide, 73.8 days. There was no significant difference ($p > 0.05$) between the biological half-lives for both compounds. However, there was a significant difference ($p < 0.05$) between the percentages of fractional retention for fast and slow components for both compounds. Thus, the hydrated indium oxide particles were retained within the body to a greater degree than the ionic indium.

Pathology. Ionic indium (LD_{50} dose) produced extensive damage to the kidney. The hydrated indium oxide (LD_{50} dose) produced extensive damage to those organs associated with the RES (liver, spleen, bone marrow). The pathological data have been previously reported in detail (*17*).

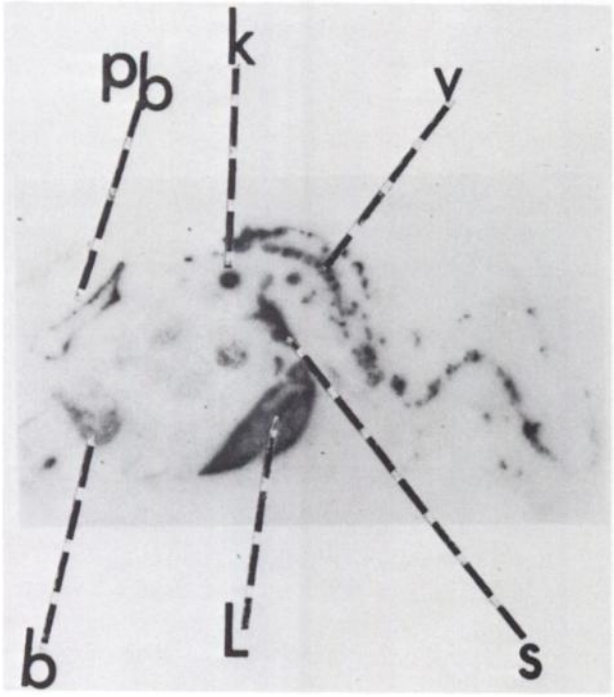

FIG. 3. Whole-body autoradiograph of ionic indium 1 day postadministration. L = liver, k = pole of kidney, s = spleen, v = vertebrae (marrow), b = bladder, pb = pelvic bone.

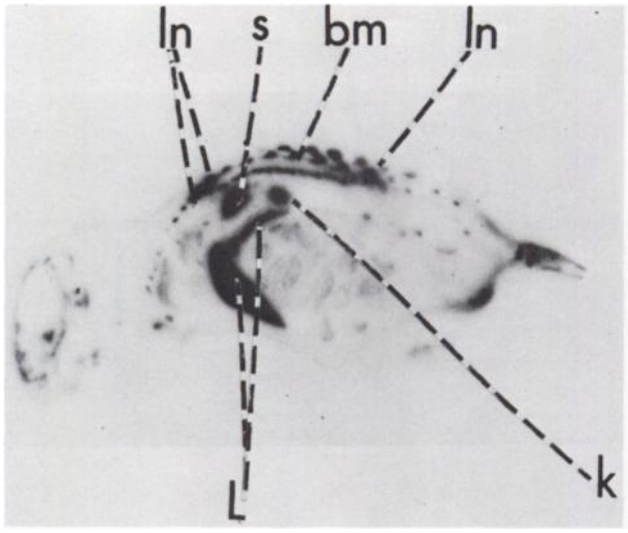

FIG. 4. Whole-body autoradiograph of colloidal hydrated indium oxide 1 day postadministration. L = liver, k = pole of kidney, ln = lymph node, s = spleen.

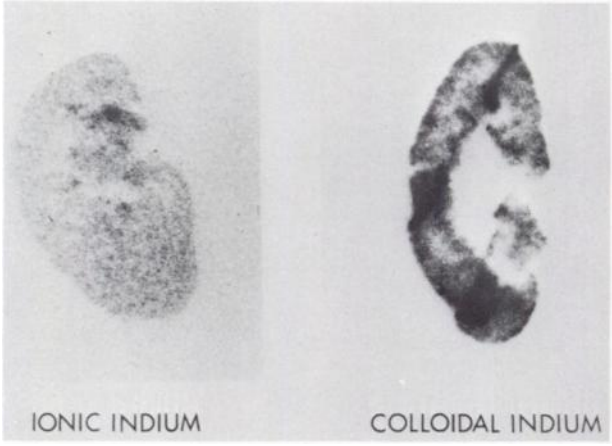

FIG. 5. Organ autoradiography; ionic and colloidal indium distribution in kidney.

Autoradiography. Whole-body autoradiographic identification of ionic indium chloride and colloidal hydrated indium oxide in adult male mice 1 day postadministration are shown on Figs. 3 and 4, respectively.

Ionic indium was localized (Fig. 3) in the liver, spleen, kidney, pelvic bone, bladder, and vertebrae (marrow). Figure 4 shows the colloidal indium distribution pattern in the liver, spleen, lymph nodes, bone marrow, and kidney.

There was homogeneous uptake of ionic and colloidal indium in the spleen and liver. However, the kidney distribution was markedly different as illus-

TABLE 2. FRACTIONAL RETENTION; BIOLOGICAL HALF TIME AND EXCRETION DATA FOR IONIC INDIUM AND HYDRATED INDIUM OXIDE

Ionic indium					Hydrated indium oxide				
Dose Tracer	fast 31%	$T_{1/2}$b 1.9d	slow 69%	$T_{1/2}$b 69d	Dose Tracer	fast 18%	$T_{1/2}$b 2.0	slow 82%	$T_{1/2}$b 73.8d
	Excretion					Excretion			
Days	Feces		Urine		Days	Feces		Urine	
0–1	6.73		28.11		0–1	7.61		2.53	
1–2	6.66		3.94		1–2	5.28		0.82	
2–3	4.72		4.23		2–3	4.64		0.91	
3–6	7.08		5.43		3–6	8.32		1.29	
6–8	1.05		2.89		6–8	6.08		0.92	
9–10	1.34		2.47		9–10	3.42		0.92	
10–13	2.41		1.05		10–13	4.56		0.23	
13–16	1.76		2.46		13–16	4.03		0.36	
16–20	1.34		1.11		16–20	3.13		1.05	
20–23	0.57		0.11		20–23	2.20		0.27	
23–30	0.92		0.66		23–30	3.84		0.38	
Total	34.58		52.46		Total	53.11		9.68	

trated in Fig. 5. The ionic compound concentrated in cortex and medulla whereas the colloidal agent concentrated only in the cortex at 24 hr.

DISCUSSION

Since the discovery of the process of phagocytosis by Metchnikoff in 1883 (*20*), emphasis has been placed on its protective function. The present experiments suggest that at times the phagocytic function of the reticuloendothelial system (RES) may result in deleterious effects. In studies of the relative toxicity of two chemical forms of the heavy metal indium it was found that the particulate form, which is rapidly taken up by the cells of the RES, was 40 times more toxic on a weight basis than the ionic form which was primarily bound to plasma proteins. While it is possible that the toxicity of the particulate form would have been even greater were it not for the phagocytic function of the RES, it is also possible that the process of phagocytosis, by localizing the material in high concentrations in the liver and other RES organs, enhanced the toxicity of the element. In fact, the toxicity of indium has been reduced by prior blockade of the RES (*21*).

The toxic action of inorganic ionic indium in the kidney is probably the cause of death, and, in this respect, it resembles inorganic mercury (*22,23*). The lethal (LD_{50}) range for indium chloride in rats, rabbits, and dogs is cited in the literature to be 0.33–3.6 mg of indium per kilogram of body weight (*9*). In mice, we found an LD_{50} value of 12.6 mg of indium per kilogram of body weight. Previous studies were concerned with indium chelated with citrate at a pH of approximately 5.0. In the present study the indium was ionic, not chelated, and at a pH of 3.0. The difference in lethalities could also be due to species variation.

The lethality of the hydrated indium oxide has not been investigated previously. After intravenous injection the colloidal particles were rapidly cleared from the blood by the RES. Nonstabilized preparations of insoluble indium sesquioxide (*6*) were less toxic to rabbits than the stabilized hydrated indium oxide that was more readily phagocytized by the RES. Before death, the mice bled from the ears, nose, and mouth. At autopsy, extensive hemorrhaging was found in the large intestine and liver and the latter contained quantities of fibrin thrombi (*17*).

Based on these lethality studies, the clinical safety factor for both ^{113m}In and ^{111}In is considerable ($\approx 10^5$).

Ionic indium chloride, when injected at pH 3.0, became protein bound after injection. Subsequent translocation was to the kidney, liver, and spleen and on a per gram basis the femur (bone marrow) contained a significant amount at Day 1. This suggests the possibility of using ^{111}In ($T_{1/2}$ 2.8 days) labeled ionic indium chloride as a bone marrow scanning agent approximately 24 hr postadministration. Our studies did not determine whether the indium was cleared by the bone marrow in the colloidal state or in a chemical form closely associated with ferrokinetics. Previous studies suggest the indium to be distributed in erythroid marrow (*13*).

Colloidal hydrated indium oxide was rapidly cleared from the blood by the RES, primarily by the liver and spleen. The feces was the main route of excretion for hydrated indium oxide.

The cortical distribution of colloidal indium in the kidney (Fig. 5) suggests capillary blockage and/or phagocytic activity in this region. This may be an important factor in calculating the adsorbed dose to the kidney. However, we do not know whether similar results will be observed in man after colloidal indium administration.

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REFERENCES

1. STERN HS, GOODWIN DA, SCHEFFEL U, et al: In^{113m} for blood pool and brain scanning. *Nucleonics* 24: No 2, 65–69, 1967

2. STERN HS, GOODWIN DA, WAGNER HN, et al: In^{113m}, a short-lived isotope for lung scanning. *Nucleonics* 24: No 10, 57–59, 1966

3. GOODWIN DA, STERN HS, WAGNER HN: A new radiopharmaceutical for liver scanning. *Nucleonics* 24: No 11, 65–66, 1966

4. STOKINGER HE: The metals. In *Industrial Hygiene and Toxicology,* vol II, Patty F, ed, New York, Interscience, 1966, pp 1048–1052

5. HODGMAN CD: *Handbook of Chemistry and Physics,* 44th ed, Cleveland, The Chemical Rubber Co, 1963

6. SUNDERMAN DH, TOWNLEY CW: *The Radiochemistry of Indium.* Nuclear Sciences Series, National Academy of Sciences Series, National Academy of Sciences, National Research Council, NAS-NA-3014. USAEC, May 1960

7. MCCORD CP, MEEK SF, HARROLD GC, et al: The physiologic properties of indium and its compounds. *J Ind Hyg Toxicol* 24: 243–258, 1942

8. DOWNS WL, SCOTT JK, STEADMAN LT, et al: *The Toxicity of Indium.* USAEC Research and Development Report, UR-558, 1959

9. HARROLD GC, MEEK SF, WHITMAN N, et al: The physiologic properties of indium and its compounds, II. *J Ind Hyg Toxicol* 25: 233–237, 1943

10. SMITH GA, THOMAS RC, BLACK B, et al: *The Metabolism of Indium 114m Administered in the Rat by Intratracheal Intubation.* Report UR-500. University of Rochester, Atomic Energy Project, 1957

11. SMITH GA, SCOTT JK: *Metabolism of Indium-II. The Distribution and Excretion of Indium-114m Administered to the Rat by Subcutaneous Injection.* Report UR-507, University of Rochester, Atomic Energy Project, 1957

12. MARROW PF, GIBB FR, CLOUTIER R, et al: *Fate of Indium Sesquioxide and of Indium-114m Trichloride Hydrolysate Following Inhalation in Rats.* Report UR-508, University of Rochester, Atomic Energy Project, 1958

13. LILIEN DL, BERGER HG, ANDERSON DP, et al: Indium-111 chloride: a new agent for bone marrow imaging. Works in progress section, Soc of Nucl Med, Boston, Mass, 1972

14. HOSAIN F, MCINTYRE P, POULOSE K, et al: Binding of trace amounts of ionic indium to plasma transferrin. *Biochem Biophys Acta* 127: 66–71, 1969

15. MOELLER T: Contributions to the chemistry of indium-115; an electrometric study of the precipitation of hydrous indium hydroxide. *J Am Chem Soc* 63: 2625–2628, 1941

16. MILLER LC, TAINTER ML: Estimation of the LD-50 and its error by means of logarithmic probit graph paper. *Proc Soc Exp Biol Med* 57: 261–264, 1944

17. CASTRONOVO FP, WAGNER HN: Factors affecting the toxicity of the element indium. *Br J Exp Pathol* 52: 543–559, 1971

18. ULLBERG S: Peaceful uses of atomic energy. *Proc Soc International Conference* 24: 248, 1958

19. WATKINS J, TEE DEH, WANG M, et al: Isolation and characterization of mouse transferrins. *Biochem Biophys* Acta 127: 66–71, 1966

20. METCHNIKOFF E: Untersuchung uber die Mesodermulen Phagocyten einiger Wirbeltiere. *Biologish Centralblatt* 18: 560–573, 1883

21. EVDOKIMOFF V, WAGNER HN: Reduction of indium toxicity by blockage of the reticuloendothelial systems. *RES J Reticuloendothelial Soc* 11: 599–603, 1972

22. HARVEY SC: Heavy metals. In *The Pharmocological Basis of Therapeutics.* Goodman L, Gilman A, eds, New York, Macmillan, 1965, pp 943–975

23. BICKERS JN, BRESLER EH, WEINBERGER R: Inhibition of succinic dehydrogenase by mercury. *J Pharmacol Exp Ther* 128: 283–287, 1960