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IRON HYDROXIDE PARTICLE RETENTION IN PRIMATE LUNGS

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The retention and possible toxicity of iron hydroxide particles used for lung scanning were investigated by incorporating ^{59}Fe and injecting the left pulmonary arteries of three monkeys retaining the right lungs for control. Washout rates from external counts were obtained using a special collimator device and the animals were sacrificed at 4–7 months. The lungs were assayed for residual radioiron and examined microscopically for iron staining and tissue damage.

A three-exponential washout curve with approximate $T_{1/2}$'s of 8 hr, 6 days, and 100 days was obtained. These compartments contained 42–54%, 21–39%, and 15–38%, respectively, of the injected dose. Projection of the slow rate suggests 1–4% would remain in the lung after a year. Microscopic examination showed particles up to 8 microns confined to macrophages, with no evidence of tissue damage. It is unlikely that iron hydroxide lung scans pose any risk of long-term toxicity.

Suspended particles of rust-colored iron hydroxide readily form in iron salt solutions upon addition of sodium hydroxide. Since their introduction by Stern in 1966 (*1*), these particles have had strong appeal to investigators for lung scanning. During formation they firmly bind trace amounts of indium, gallium, or technetium. Their preparation from common laboratory reagents requires less than 15 min and the addition of gelatin makes them stable for an entire day. At our institution, over 549 human injections have caused only two reactions: one patient had flushing for 5 min and another complained of burning at the injection site. However, a major reaction remains an accepted hazard as with most intravenous particulate agents. Liver and spleen are faintly visualized in some cases but this usually helps rather than hinders diagnosis. With such favorable qualities, one would expect this inexpensive agent to be more widely used. However, there is an unresolved problem of long-term effect on the lungs.

Five different investigators in the first few weeks after injecting mice and rabbits found no lung damage. They used equivalents up to 10,000 times the 2-mg maximum dose used in man (*1–5*). An exception to this is the report of Rhodes, et al (*6*) that 10 mg/kg produced microscopic lung damage in 83% of mice. This dose is equivalent to 500 times the human dose. Most investigators agree that about 80% of activity is deposited immediately in the lung and washes out with a fast compartment of half-life 1–24 hr and a slow compartment of half-life 40 days–1 year. Estimates of the amount of iron in the slow compartment range from 10% (*5*) to 48% (*4*). In the 48% study, despite the high retention, mice sacrificed at 42 days showed no tissue damage (*4*). To determine how long the iron is finally retained and whether it is harmful, we undertook an extended study in monkeys.

METHOD

Three adult male rhesus monkeys (Macaca mullata) were subjected to right heart catheterization in order to inject the left pulmonary artery, retaining the right lung as a control. The animals were sedated with intramuscular phencyclidine hydrochloride for ease of handling. From a previous ^{99m}Tc-iron hydroxide lung scan, it was determined in Monkey No. 1 that 5-cm circles below each clavicle, symmetrically placed, would overlie comparable volumes of lung and chest wall.

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On the day of injection, eluate from a decayed technetium generator and 20–40 μCi of ^{59}FeCl were incorporated into iron hydroxide aggregates prepared by the method of Bruno, et al (*7*). The particles were then washed with saline to remove free ^{59}Fe and resuspended. They ranged from 3–120 microns in size and were in the ferric or oxidized form. An aliquot was taken for standards and the remainder slowly injected into the left pulmonary artery. The elemental iron deposited in the left lung was 1.28 mg for Monkey No. 1, 0.70 mg for Monkey No. 2, and 0.74 mg for Monkey No. 3. For Monkey No. 1, this was 0.14 mg/kg or five times the human maximum dose.

External monitoring was done with a 3-in. sodium iodide crystal shielded by 3 in. of lead. Shield penetration from the 1.1 and 1.3 MeV radiations was estimated by counting each area with and without a hollowed lead cone in the collimator mouth (Fig. 1). This cone interferes only with photons arising from the tissue of interest and not with photons penetrating the shield. The true counting rate is calculated by subtracting the counting rate with the cone from the counting rate without the cone and dividing by one minus the transmission factor (T) of this cone:

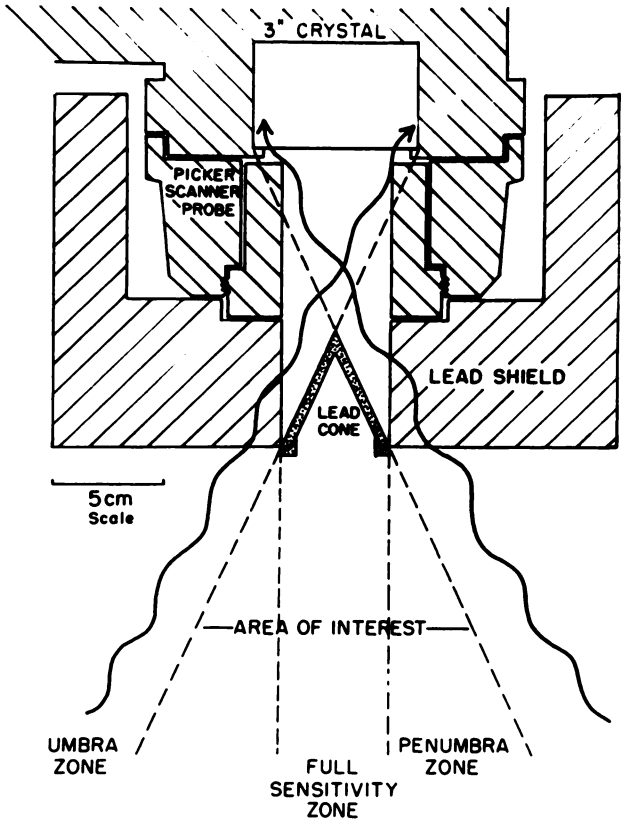

FIG. 1. Diagram of high-energy gamma collimator with lead cone attenuating radiation from area of interest but not from region.

1. CPM_1 (without cone) = Instrument Bkg + Counts from Area of Interest + Penetration Counts
2. CPM_2 (with cone) = Instrument Bkg with cone + (T × Counts from area of Interest) + Penetration Counts

Subtraction of 2 from 1 and simplification yields:

Counts from Area of Interest =

$$\frac{CPM_1 - CPM_2 - Bkg_1 + Bkg_2}{1 - T}$$

This method (*8*) is accurate down to 5% of the isotope left in the area of interest with the other 95% causing shield penetration. At that point there is considerable fluctuation in serial counts. A final correction was made for marrow and red cell radioiron by subtracting right from left lung counting rates. This gave the test lung activity remaining from the original particle deposition whether the material was already solubilized and phagocytosed or still particulate. This probe monitored one-third of each lung, as indicated by the external counting rate immediately after injection compared with the standard, and by the counting rate at the end of the curve compared with the excised lung. Initial low liver counts suggested that almost no particles passed through the lung.

The animals were sacrificed at 4, 5, and 7 months. Under anesthesia, we quickly opened the chest and clamped the lung roots to prevent hemorrhages. The excised lungs were perfused with saline under less than 20-cm H_2O pressure to remove blood, fume fixed by the method of Hentel and Longfield (*9,10*), and assayed in a large volume counter. They were also autoradiographed and examined microscopically for tissue damage and iron staining. The microscopic estimate was done by blind comparison of test and control lungs.

RESULTS

Clearance rates. Monkey No. 3 experienced inadvertent spillage of some particles into the right pulmonary artery at injection and this was probably the cause of an erratic elution curve. However, Monkeys 1 and 2 displayed smooth, almost identical "best fit" curves, suitable for analysis (Figs. 2, 3). Two aberrant points between Days 35 and 40 that fell between 1 and 2% were minimized in fitting the curve. Each curve showed three compartments. The rapid one with 42–54% of the particles washed out with a $T_{1/2}$ of 8–9 hr. The intermediate one with 21–39% washed out with a $T_{1/2}$ of 3–6 days. The slow compartment contained 15–38% and disap-

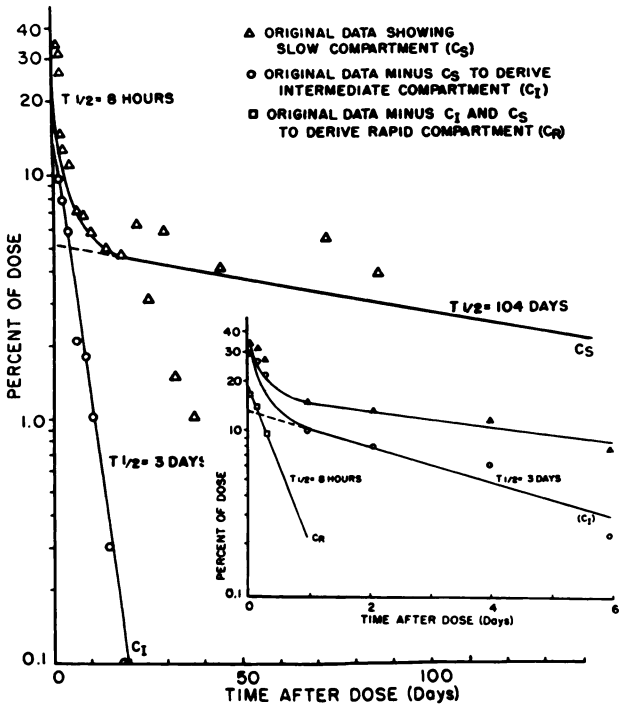

FIG. 2. Disappearance curve of ^{59}Fe-iron hydroxide particles from upper lung area (Monkey No. 1). Expansion of first 6 days shown as insert.

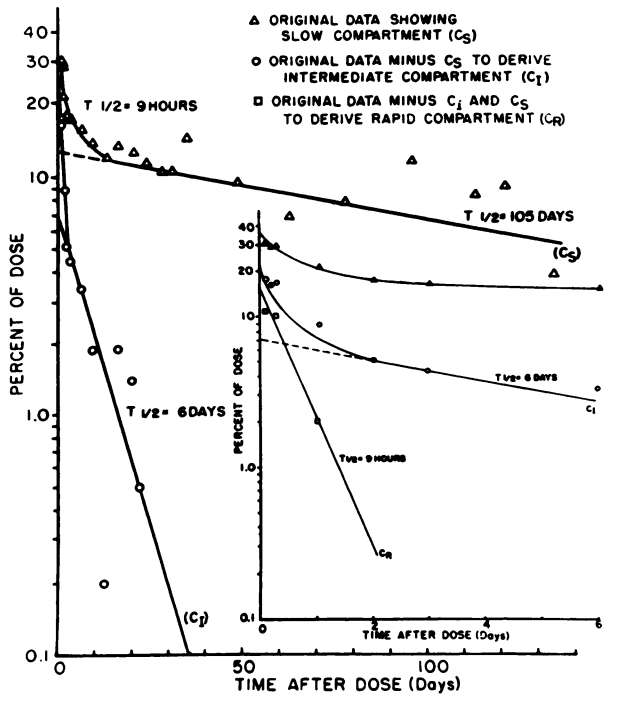

FIG. 3. Disappearance curve of ^{59}Fe-iron hydroxide particles from upper lung area (Monkey No. 2). Expansion of first 6 days shown as insert.

peared with a $T_{1/2}$ of 104–105 days. Projection of this slow rate suggests 1–4% would remain in the lung after a year.

Postmortem retention. Assuming the few minutes low pressure saline perfusion did not dislodge iron particles, the postmortem radioiron content 4–7 months after injection was 4.2, 15.3, and 14.8% of the dose. This corresponded very nearly to three times the external estimate from monitoring one-third of the lungs: 2.5, 5.3, and 6.5% (Table 1). This correlation supports the above assumption.

Particle distribution. Microscopic sections 5 microns thick were evaluated without knowledge of their identity. There was no trace of tissue damage in either the test or control lungs. Iron stains showed no visible iron in the control lungs except for a trace in Monkey No. 3, in which there had been slight spillage. All sections of the test lungs showed iron particles in the connective tissue, confined to macrophages, with a density of 1–15 deposits/1.2-mm microscopic field. No particles were present in alveoli or capillaries. Particles ranged from less than 1 up to 8 microns and none had evoked any pathologic reaction. Gross autoradiograms failed to demonstrate any hilar node concentration and they confirmed the patchy septal distribution.

TABLE 1. RETENTION OF ^{59}Fe IN THREE MONKEY LUNGS* AT 4, 5, AND 7 MONTHS AFTER INJECTION

	Weight (gm)	Net† CPM	CPM/ gm	Dose (%)
Left upper lobe				
Monkey No. 1	1.41	396	283	1.16
Monkey No. 2	1.10	1,109	1,008	1.40
Monkey No. 3	0.83	2,010	2,422	8.40
Left middle lobe				
Monkey No. 1	2.48	595	238	1.75
Monkey No. 2	2.00	3,874	1,937	5.00
Monkey No. 3	0.70	752	1,074	3.14
Left lower lobe				
Monkey No. 1	2.64	512	197	1.50
Monkey No. 2	0.98	7,085	7,230	9.20
Monkey No. 3	2.16	2,939	1,361	12.27
Total left lung				
Monkey No. 1	6.53	1,503	230	4.41
Monkey No. 2	4.08	12,068	2,958	15.60
Monkey No. 3	3.39	5,701	1,682	23.81
Right lung				
Monkey No. 1	6.78	18	8	0.16
Monkey No. 2	4.80	209	43.5	0.27
Monkey No. 3	4.28	2,150	502	8.97
Left lung minus right lung‡				
Monkey No. 1	—	1,485	—	4.25
Monkey No. 2	—	11,859	—	15.33
Monkey No. 3	—	3,551	—	14.84

* Monkeys Nos. 1, 2, and 3 were sacrificed at 4, 5, and 7 months after injection, respectively.

† Five-min counts background 135 CPM.

‡ Left lung counts (particle + free ^{59}Fe) minus right lung counts + (free ^{59}Fe) = ^{59}Fe hydroxide particles in left lung.

DISCUSSION

From this study one can conclude that ferric hydroxide particles embolized in monkey lungs are cleared at rapid, intermediate, and slow rates. The slow rate, with half-life of approximately 100 days, comprises 15–38% of the dose. This projects to 1–4% remaining in the lung at 1 year and is in excellent agreement with the value of 3% remaining in mice lungs at 10 months obtained by Davis (*11*), using ferrous hydroxide particles. It should be noted that the present study employed the ferric form because this is the common form used clinically and is said to be cleared more slowly than the ferrous state (*11*). At diagnostic doses the slowly removed component resides in connective tissue macrophages without evidence of tissue irritation. Experience in guinea pigs cited by Bertalanffy (*12*) indicates complete lung clearance of dust particles from macrophages by 10 months. On this basis, there should be an 18-month study utilizing the long 2-year half-life of ^{55}Fe. Meanwhile, it seems unlikely that iron hydroxide lung scans pose any risk of long-term toxicity.

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REFERENCES

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

2. DAVIS MA: ^{99m}Tc-iron hydroxide aggregates. Evaluation of a new lung-scanning agent. *Radiology* 95: 347–352, 1970

3. COLOMBETTI LG, GOODWIN DA, TOGAMI E: ^{68}Ga-labeled macroaggregates for lung studies. *J Nucl Med* 11: 704–707, 1970

4. BARKER SL, GUSMANO EA, SMITH TD, et al: Retention of ^{113m}In-^{59}Fe-ferric hydroxide in the mouse. *J Nucl Med* 12: 5–7, 1971

5. GOODWIN DA: Lung retention of labeled ferric hydroxide macroaggregates used in lung scanning. *J Nucl Med* 12: 580–581, 1971

6. RHODES BA, ZOLLE I, BUCHANAN BA, et al: Radioactive albumin microspheres for studies of the pulmonary circulation. *Radiology* 92: 1453–1460, 1969

7. BRUNO FP, BROOKEMAN VA, ARBORELIUS M, et al: ^{99m}Tc-iron hydroxide aggregates for lung scanning. *J Nucl Med* 11: 134–137, 1970

8. WATTS RS, PEÑA HG: A method of correcting for collimator penetration of high energy gamma rays. *J Nucl Biol Med* (Italy) 16: 51–54, 1972

9. HENTEL W, LONGFIELD AN: A stereoscopic study of the inflated lung. *Dis Chest* 38: 357–361, 1960

10. HENTEL W, LONGFIELD AN, GORDON J: A re-evaluation of bronchiectasis using fume fixation. *Dis Chest* 41: 41–45, 1962

11. DAVIS MA: Long term retention and biologic fate of ^{99m}Tc-iron hydroxide aggregates. In *Radiopharmaceuticals and Labeled Compounds*, Vol 2, Vienna, IAEA, 1973, pp 43–63

12. BERTALANFFY FD: Respiratory tissue: structure, histophysiology, cytodynamics, part I. *Int Rev Cytol* 16: 233–318, 1964