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CHEMICAL AND BIOLOGIC PROPERTIES OF ^{111}In-PHOSPHATE FOR CISTERNOGRAPHY AND GLOMERULAR FILTRATION STUDIES

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Inorganic ^{111}In-phosphate has been evaluated for cisternography in dogs, rats, and one human volunteer for chemical and biologic stability and renal clearance. It appears to be stable and to be completely cleared by glomerular filtration, suggesting its suitability for cisternography.

Occasional adverse reactions following radionuclide cisternography with radioiodinated human serum albumin (*1,2*), the diffusibility of ^{111}In chelates (*3*), and the failure of colloidal ^{111}In-phosphates for CSF studies (*4*) prompted us to explore other radiopharmaceuticals.

A small, molecular, noncolloidal, inert complex like ^{111}In-phosphate with a physical half-life of 2.81 days may lend itself well to cisternography.

MATERIALS AND METHODS

The sterile and pyrogen-free ^{111}In (Diagnostic Isotopes) was obtained in the trichloride form in dilute hydrochloric acid (pH 1.5–2.0). The activity of 1 mCi/ml was in the carrier-free state. Potassium phosphate monobasic sodium hydroxide buffer (0.05 *M*) pH 7.4 (Fisher Scientific) was sterilized by autoclaving and then passed through an 0.05-micron Millipore filter. This stock solution was prepared in bulk and kept refrigerated.

An aliquot of $^{111}InCl_3$ was drawn up in a sterile 10-ml syringe and then 4 ml of phosphate buffer were drawn into the same syringe. After mixing, this material was filtered through an 0.05-micron Millipore filter into a sterile multi-injection vial for dispensing. This solution had a final pH of 7.1–7.2. The yield of ^{111}In-phosphate that passed through the 0.05-micron filter was 100%. No trace of filterable ^{111}In-phosphate remained on the filter.

Chemical properties. Chromatography on Whatman No. 3 MM paper developed with ethanol/water (60/40) showed that $^{111}InCl_3$ remained at the origin and that ^{111}In-phosphate moved to an R_f value of 0.68. There was no trace of free $^{111}InCl_3$ at the origin (Fig. 1).

Further evidence of this quantitative conversion was obtained by gel filtration analysis. A column of Sephadex 50 (9 × 240 mm), (Sephadex G-50, fine, 20–80 micron size, approximate exclusion limit mol wt 10,000, supplied by Pharmacia Laboratories) eluted with physiologic saline solution at a rate of 0.5 ml/min, showed a single peak at 14.6 ml. This peak represented 100% of the activity placed on

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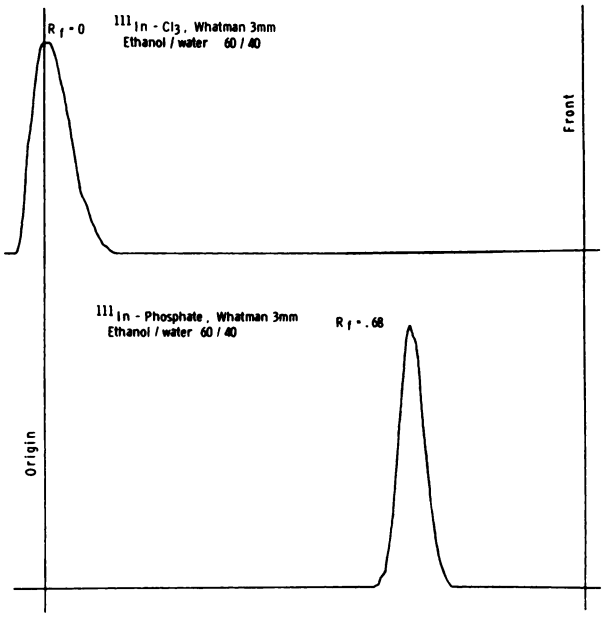

FIG. 1. Ascending chromatograms of $^{111}InCl_3$ and ^{111}In-phosphate.

TABLE 1. SIMULTANEOUS CLEARANCE OF ^{14}C-INULIN AND ^{111}In-PHOSPHATE IN DOG

Dog No. (weight)	Clearance of ^{14}C-inulin (ml/min)	Clearance of ^{111}In-phosphate (ml/min)
1 (21 kg)		
1	50.1	48.9
2	47.8	47.8
3	45.0	47.6
4	39.0	42.6
Mean	45.5	46.6
s.d.	±4.1	±2.6
2 (23 kg)		
1	81.8	78.1
2	75.2	70.7
3	60.8	64.3
4	64.4	64.1
Mean	70.4	69.3
s.d.	±8.4	±5.7
3 (24 kg)		
1	56.7	57.9
2	66.0	68.0
3	64.9	66.2
4	70.0	71.5
Mean	64.4	65.9
s.d.	±4.8	±5.0
Clearance ratio	0.992	

the column; $^{111}InCl_3$ remained firmly bound to Sephadex 50 and no activity could be eluted with saline solution.

Chemical and biologic stability. Chromatography of stock solutions of ^{111}In-phosphate showed no change from the freshly prepared reagent over a 2-week period.

Physiologic stability of this material was demonstrated by the intravenous injection of 1 ml of ^{111}In-phosphate solution diluted to 10 μCi/ml with saline solution into ten rats. Urine was collected over a 24-hr period. Chromatography of urine samples showed a single peak identical with the injected ^{111}In-phosphate.

Biologic half-life. In the same ten rats we found that 74–88% of the injected activity was excreted in the urine in 3 hr and 97–100.5% in 24 hr. In two greyhounds of 21 kg and 24 kg, 72 to 80% was detected in the urine 3 hr after an intravenous injection of 50 μCi of ^{111}In-phosphate.

In one human volunteer, negligible activity could be detected by whole-body scanning 24 hr after an intravenous injection of 500 μCi of ^{111}In-phosphate.

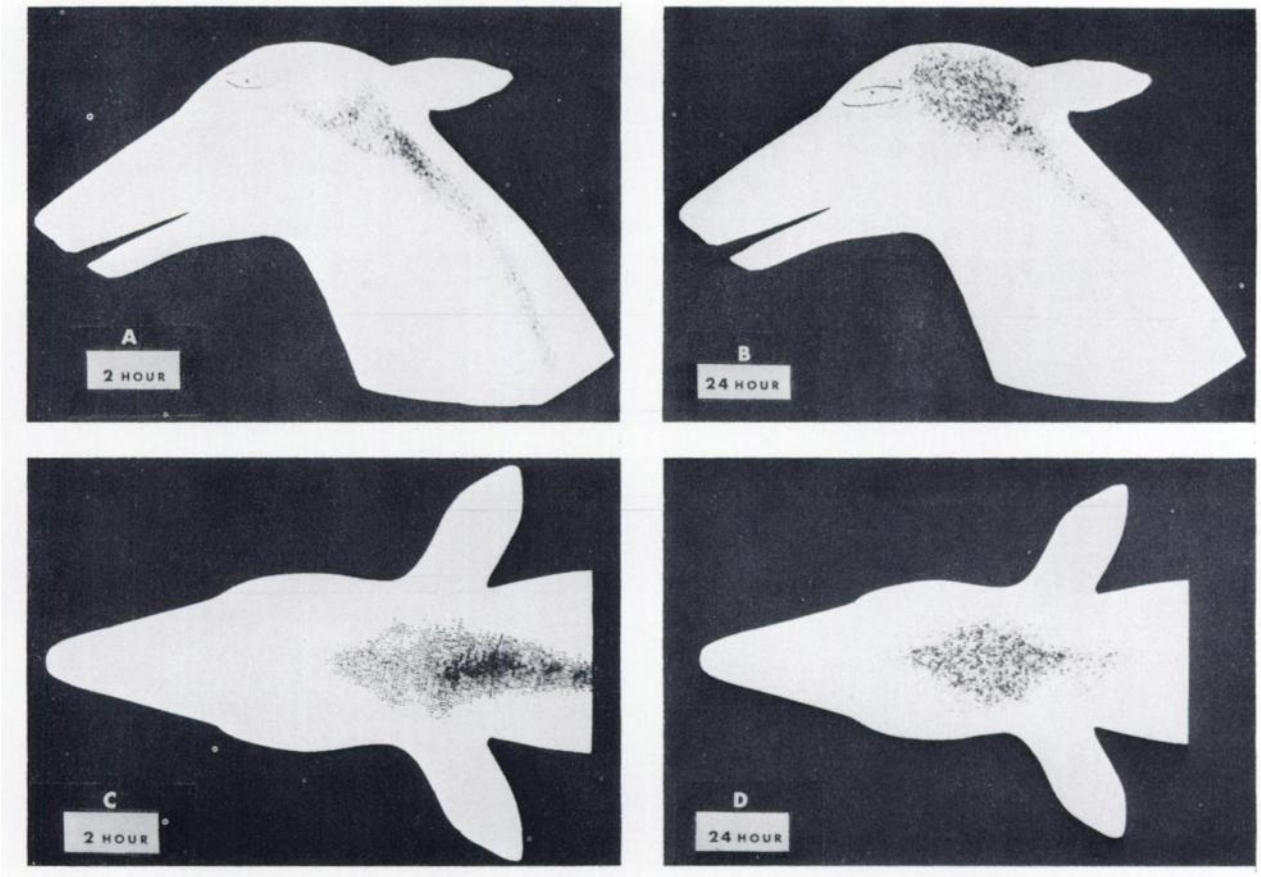

FIG. 2. Left lateral (A and B) and vertex (C and D) views of normal cisternogram in dog 2 and 24 hr after injection of 500 μCi of ^{111}In-phosphate.

Blood clearance in two anesthetized dogs given 50 μCi of ^{111}In phosphate intravenously gave half-times of 28 and 30 min and in the single experiment in man the half-time blood clearance was 22 min.

Glomerular filtration. Proof that the renal clearance of ^{111}In-phosphate was by glomerular filtration was demonstrated by measuring the clearance by the continuous infusion method in three anesthetized greyhounds. Fifty microcuries of ^{111}In-phosphate and 50 μCi of ^{14}C-inulin were added to 50 ml of a 10% inulin solution. Assay of the ^{111}In was done in a 3-in. well counter. These samples were then combusted in a Packard sample oxidizer and the $^{14}CO_2$ was analyzed by liquid scintillation counting. The resulting clearance rates for ^{111}In-phosphate and ^{14}C-inulin were identical (Table 1).

Since ^{111}In-phosphate is biologically stable and completely cleared by renal excretion, this radiopharmaceutical was used for experimental cisternography in five anesthetized greyhounds. Indium-111-phosphate (500 μCi in 2.5 ml) was injected into the subarachnoid space through the atlanto-occipital membrane. This site of injection was technically easier than the difficult lumbar puncture.

Rectilinear scans on these animals at 2- and 24-hr intervals were taken (Fig. 2) (Picker Nuclear 5-in. magnascanner set on narrow window for the 247-keV peak of ^{111}In). In 24 hr ^{111}In-phosphate clearly outlined the entire cerebrospinal fluid system. The animals were observed for 1 week after injection and no adverse clinical effects were seen.

DISCUSSION

Indium-111-phosphate appears to behave as a soluble neutral inorganic compound that is predominantly of the covalent type at the pH range used in this study. The lack of ionization at such dilute concentrations is demonstrated by the fact that ion-exchange resins do not remove ^{111}In from solution. It is surprising to see such stable bonding of inorganic ions in the presence of complete solubility. We know from gel filtration analysis that the molecular weight is about 200–600 and that the compound is stable at physiologic pH in vitro as well as in vivo. The chemical structure of ^{111}In-phosphate remains to be identified.

The sterilized phosphate buffer can be prepared in bulk and pyrogen testing can be done far in advance of preparing the radiopharmaceutical. Since the ^{111}In used is carrier-free, the ^{111}In-phosphate can be injected in very small amounts because the bulk of the injection is phosphate buffer. Toxic effects of 1 ml of 0.05 *M* phosphate buffer on the central nervous system are highly unlikely but no systematic study had been made by us. Similarly, a study of the clearance rate of ^{111}In-phosphate from the spinal fluid space is not yet available.

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