JOURNAL OF NUCLEAR MEDICINE 6:287-291, 1965

Purification of Radioactive Sodium O-Iodohippurate Using Gel Filtration with DEAE-Sephadex[1]

Donald W. Brown, Norma J. Phelps and Darwin L. Palmer[2]

Denver

To perform renal clearance studies with radioactive o-iodohippurate and obtain results which are comparable to those obtained with para-amino-hippurate, it is necessary that the material be of the highest possible radiopharmaceutical purity (1-4). Although manufacturers have made great strides in removing radioactive impurities from commercially available o-iodohippurate, shipments still usually contain significant contaminants. Using gel filtration with DEAE-Sephadex, a new combination of anion exchange resin and gel, we have developed a simple and reliable method of removing these contaminants, leaving the hippurate suitable for intravenous administration.

METHOD

Six grams of commercial A-25 Coarse DEAE-Sephadex are washed three times in 150 cc aliquots of sterile pyrogen-free normal saline (NS). A 20 × 1.3 cm piece of glass tubing is stoppered at the lower end with a one-hole rubber stopper fitted with tubing and a clamp. Above this stopper is placed a glass wool plug and above the plug a 1 cm layer of tiny beads. The tube is then filled with NS which is allowed to start dripping from the lower end while the wet-washed DEAE-Sephadex is poured in from the top. Great care is taken to insure homogeneity throughout the column and to avoid the formation of air bubbles. When the gel layer is within one inch of the upper end of the glass tube, a

[1]This work was supported by USPHS Research Grant No. HE-09112-01.

[2]From the Section of Nuclear Medicine, Department of Radiology, University of Colorado Medical Center, Denver, Colorado.

circular piece of filter paper is placed on the top of the gel, the glass tube is filled to the top with NS and stoppered with a one-hole rubber stopper. This stopper is connected with tubing to a 1000 cc bottle of NS placed about two feet above the column. A level is used to assure a true vertical position. Saline is allowed to wash through the column for about one hour before use.

To load the column, the saline is allowed to drain down to the level of the filter paper. Then the o-iodohippurate solution is carefully layered on top and it too, is allowed to run down to the level of the filter paper. Next, the column is filled to the top with NS, the rate of flow adjusted and sample collection begun.

We have found a flow rate of 1 cc per minute to be very satisfactory. About 120 5 cc samples are collected and these are counted in a scintillation well counter. When not in use, the column is stored in a refrigerator at 5° C.

All of the equipment that comes in contact with the NS and hippurate is sterilized before use. After gel filtration, the material from the two test tubes containing the highest concentration of peak B (see below) is transferred to a sterile vial through a sterilizing 45 millimicron-size millipore filter then sterility and pyrogen tested before use.

RESULTS

Typical separations are demonstrated in Figs. 1 and 2. Three peaks are

TABLE

Shipment	*Days**	*Fraction*		
		A (%)	*B* (%)	*C* (%)
X	1	0.6	98.9	0.5
	13	0.8	94.8	4.4
	18	0.7	95.3	4.0
Y	6	0.6	98.8	0.6
	13	0.7	98.5	0.8
	20	0.7	98.6	0.7
	27	0.7	98.4	0.9
Z	1	3.4	95.3	1.3
	7	3.3	94.5	2.2

Fractionation of representative shipments from 3 manufacturers of O-iodohippurate with DEAE-Sephadex

*Days from receipt of shipment.

consistently found and are labeled A, B, and C. Contaminants A comes through almost at once, indicating that it is probably very large in particle size. Contaminant C comes through late and appears to represent free iodide. This assumption is based on the fact that when free sodium ^{125}I is mixed with ^{131}I labeled o-iodohippurate and the mixture fractionated, the ^{125}I ion comes through with fraction C of the o-iodohippurate.

A surprising difference was found in the percent of contaminants A and C in shipments from different commercial suppliers of ^{131}I o-iodohippurate. Repre-

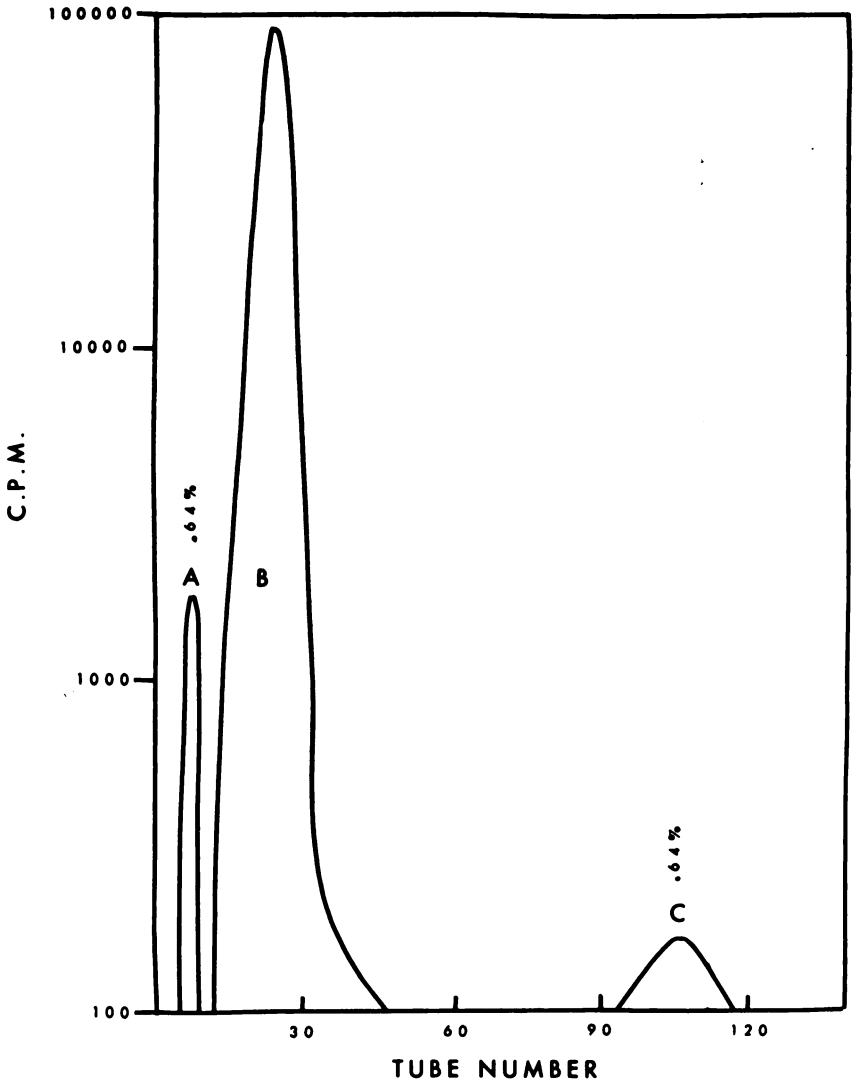

Fig. 1. DEAE-Sephadex gel filtration separation of ^{131}I O-iodohippurate (fraction B). Fraction A is a large particle contaminant and fraction C, free ^{131}I.

sentative fractionations from different shipments as well as measurements of their stability with time are presented in the Table.

The results of the above studies are essentially in agreement with those of others (3, 5, 6) who used paper chromatography to perform the fractionation. Our method has the advantage of producing the purified hippurate fraction in suitable form for parenteral administration.

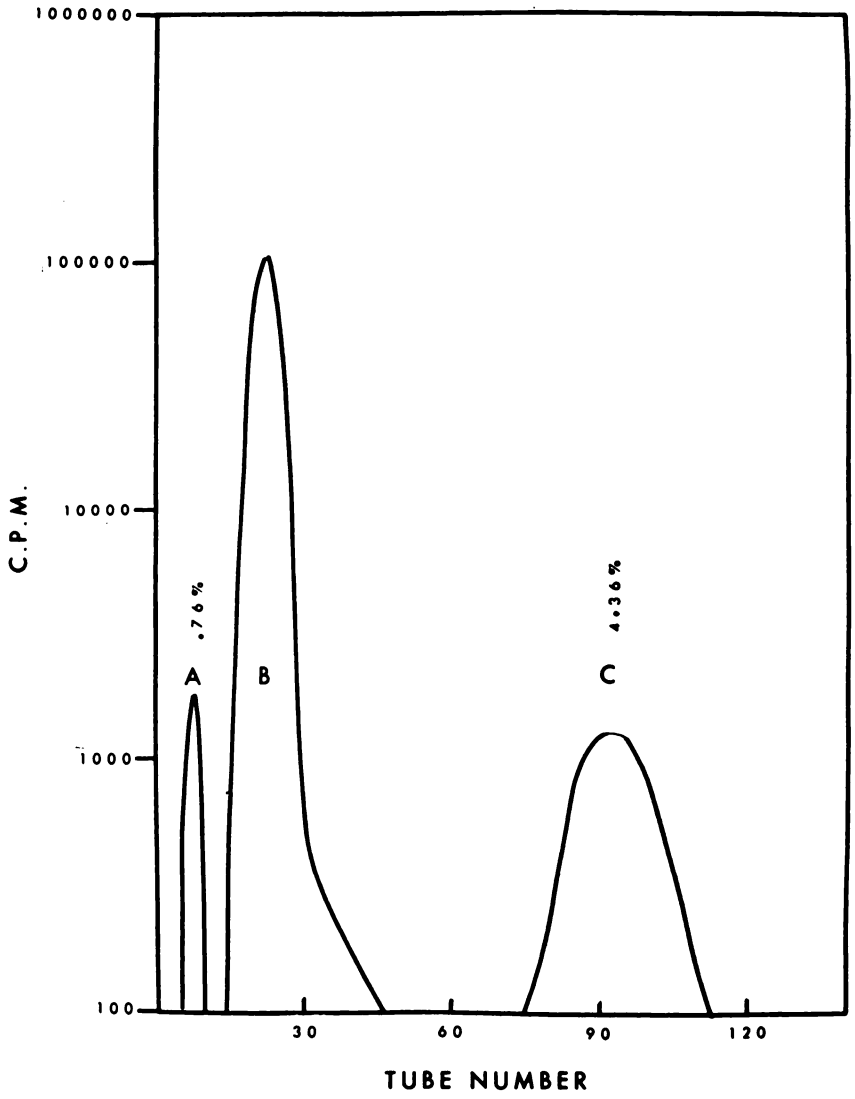

Fig. 2. Fractionation of a shipment of ^{131}I O-iodohippurate from a different supplier. As much as 5 per cent contamination with either fraction A or C was not uncommon.

SUMMARY

A simple method of separating radioactive impurities from ^{131}I labeled o-iodohippurate using gel filtration with DEAE-Sephadex is presented.

REFERENCES

1. BLAUFOX, M. D., FROHMULLER, H. G., CAMPBELL, J. C., UTZ, D. C., ORVIX, A. L. AND OWEN, C. A., JR.: A Simplified Method of Estimating Renal Function with Iodohippurate ^{131}I. *J. Surg. Research* 3:122 (March) 1963.

2. GREENLAW, R. H., AND HUDGINS, W. D.: Correlation of Renal Clearance Techniques in Dogs: Para-Aminohippuric Acid Compared with Radio-Iodinated Hippuran, *J. Nuc. Med.* 5:453 (June) 1964.

3. MESCHAN, I., HOSICK, T., SCHMID, H. AND WATTS, F. C.: Variability in Renal Clearance Rate Studies Using Fresh $OI^{131}HA$, Purified Product, and Stored Product. *J. Nuc. Med.* 4:70 (January) 1963.

4. TAUXE, W. N., BURBANK, M. K., MAHER, F. T. AND HUNT, J. C.: Renal Clearances of Radioactive Ortho-Iodo-Hippurate and Diatrizoate. *Proc. Staff Meetings of the Mayo Clinic* 39:761 (Oct.) 1964.

5. ANGHILERI, L. J.: A Chromatographic Study of the Stability of $Iodine^{131}$.

6. BURBANK, M. K., TAUXE, W. N. MAHER, F. T. AND HUNT, J. C.: Evaluation of Radioiodinated Hippuran for the Estimation of Renal Plasma Flow, *Proc. Staff Meetings of the Mayo Clinic* 36:372 (July) 1961.