International Journal of Applied Radiation and Isotopes, 1961, Vol. 12, pp. 146–147. Pergamon Press Ltd. Printed in Northern Ireland

A Simplified Method for Preparing I^{131}-Labelled Hippuran*

(*Received* 17 *October* 1961)

RADIOACTIVE renograms are the records obtained when externally situated radiation detectors are used to follow the uptake and excretion by individual kidneys of a suitable test substance administered intravenously. The procedure promises to be of considerable potential value as a simple clinical test, particularly in hypertensive patients who might have unilateral renal disease. The original test substance used for this purpose was I^{131}-labelled diodrast,[1,2] but uptake by the liver caused technical difficulties. Other substances have been tried,[3] but it now appears that I^{131}-labelled *o*-iodohippurate (Hippuran) is the ideal material since it is cleared from the plasma by the kidneys specifically[4]. Moreover, it gives results which do not differ greatly from those obtained with *p*-amino hippurate when it is used in the conventional manner for the determination of effective renal plasma flow[5]. If the kidneys are regarded as the target tissue, a tracer dose of 5 μc of I^{131} Hippuran in a normal person involves an absorbed radiation dose of 1–2 mrads; this is some two orders of magnitude smaller than that obtaining with Rb^{86}, the use of which has recently been suggested for the detection of unilateral renal artery stenosis[6].

I^{131}-labelled Hippuran is now commercially available but is somewhat expensive, the more so if the user is remote from a source of supply so that air freight charges are incurred. On the other hand, most hospitals which are equipped for radioisotope work normally carry stocks of I^{131} so that the availability of a simple, rapid and inexpensive method for preparing I^{131} Hippuran for clinical use can result in considerable economies and greater convenience. The methods described originally[7] make appreciable demands on time, skill and facilities. It has been found possible to simplify the exchange method so that these demands are substantially reduced.

The stock solutions used are:

sodium *o*-iodohippurate dihydrate (180 mg/ml)
0·01 M and 0·1 M potassium iodide
0·005 M potassium iodate
N and N/10 sodium hydroxide
N and N/10 hydrochloric acid
N/10 sodium sulphite or metabisulphite.

The required amount of carrier-free I^{131}(1–2 mc) in minimal volume is placed in a 8–10 ml capacity tube which should have a ground glass stopper and hooks for retaining springs. 0·1 ml of 0·01 M KI solution is added; 0·1 ml of N/10 HCl and 0·1 ml of KIO_3. If the radioiodine solution contains reducing agent, additional iodate should be added until the brown colour of the liberated elementary iodine is apparent. 2 ml of Hippuran solution are added, and the pH of the mixture checked with narrow range indicator paper. It is adjusted to pH 5·8–6·0 if necessary by the addition of one or two drops of N/10 HCl or NaOH. The stopper is then inserted, held in position with springs or rubber bands; and the tube is left to heat in a boiling water bath for two hours.

The tube is cooled with tap water, 0·1 ml of N HCl are added; and the resulting brown colour removed by the dropwise addition of sodium sulphite solution. About 1 ml of 0·1 M KaI is then added followed by 1 ml of N HCl. The tube is cooled in ice water for 5 min and then centrifuged for about 30 sec after which the supernatant liquid is removed.

The *o*-iodohippuric acid precipitate is dissolved in 1 ml of N NaOH and 2–3 ml of water. 1 ml of 0·1 M KI is again added, and 1·5 ml of N HCl. After cooling in ice water, the precipitate is spun down, the supernatant removed and the acid redissolved in 1 ml of N NaOH.

If the material is required for quantitative work, in which case it is necessary to remove as much as possible of the inorganic I^{131}, the above purification step can be repeated. The presence of *inactive* iodide is not important. The solubility of *o*-iodohippuric acid in N/10 HCl is about 3·8 mg/ml at 4°C, so that about 5 per cent of the material may be lost each time this step is repeated.

Finally, the solution is diluted with a few ml of water, neutralized with N HCl using wide range indicator paper; and further diluted with pyrogen free isotonic saline to give a convenient concentration of 5–10 μc/ml before dispensing into suitable dose bottles. Sterilization is effected by autoclaving at 15 lb/in^2 steam pressure for 15 min in a pressure cooker.

The radioactive yield is between 80 and 90 per cent. The specific activity of the preparation is adequate as evidenced by the clinical results obtained in several hospitals, these results being consistent with those published by workers in the U.S.A.

Acknowledgments—We are indebted to PROFESSOR H. GOTTA and DR. R. L. CERIANI for referring patients for investigation and providing facilities for clinical studies in the Clinicas Hospital, Buenos Aires. We also extend our thanks to the medical students in the Clinicas Hospital who volunteered as normal controls, and to ING. C. C. PAPADOPULOS and DR. J. VARELA of the Isotopes Section, C.N.E.A. for their encouragement and support.

This work was carried out while one of the authors (N. V.) was engaged on a Technical Assistance Mission to the Argentine Republic, under the aegis of the International Atomic Energy Agency, Vienna.

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* "Hippuran" is the registered trade name for inactive material supplied by the Mallinckrodt Chemical Works.

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