The use of liquid cation exchange in the extraction and separation of [^{14}C]neostigmine iodide and its metabolite [^{14}C]3-hydroxyphenyltrimethylammonium iodide from body fluids

Investigation of the pharmacokinetics of neostigmine and its metabolite 3-hydroxyphenyltrimethylammonium iodide (3-OH PTMA) in the rat, require the assay of these compounds in small serially collected samples of plasma and urine. Methods for their isolation and separation from urine by paper electrophoresis and chromatography have previously been described (Somani, Roberts & others, 1970). We found these methods to be unsatisfactory for the separation of the very small amounts of the compounds present, particularly in plasma.

We now describe the successful isolation and separation of both compounds by means of the selective complex which they form with sodium tetraphenyl boron (NaTPB). The technique used for extraction is based on the liquid cation exchange methods described by Fonnum (1968, 1969) to extract acetylcholine from aqueous solutions.

NaOH (0·01 ml, 5N) is added to plasma or urine (0·05 ml) containing [^{14}C]neostigmine iodide (specific activity 5·2 mCi/mmol) and [^{14}C]3-OH PTMA iodide (specific activity 10·2 mCi/mmol). Ethyl butyl ketone (0·1 ml) containing NaTPB (20 mg/ml) is then added and extraction effected by shaking, using a vortex mixer for not more than 10 s—to avoid producing an emulsion (Fonnum, 1969). After centrifugation, the upper organic phase is removed and the radioactivity assayed by liquid scintillation spectrometry. The efficiency of counting was determined by the method of the external standard channels ratio described by Barber, Bourne & Buckley (1971).

Under the alkaline conditions described above, neostigmine is well extracted from plasma and urine, but 3-OH PTMA is poorly extracted probably due to the ionization of the phenolic hydroxyl group producing an anionic site. If the addition of alkali is omitted, both compounds form extractable complexes with NaTPB so the method can be applied to the isolation of the individual compounds. The standard deviation of all replicate extractions was never greater than 2·5% and usually 1% or less. These results are shown in Table 1.

Table 1. *The extraction of* [^{14}C]*neostigmine or 3-OH PTMA from plasma and urine by NaTPB.*

	d/min, sample	Mean % extraction
Neostigmine		
A. Urine	74500	86
B. Plasma	26700	93
3-OH PTMA		
A. Urine	47000	98
B. Plasma	73700	96
Extraction under alkaline conditions:		
Neostigmine:		
A. Urine	51800	83
B. Plasma	26700	85
3-OH PTMA:		
A. Urine	33900	0·7
B. Plasma	73700	1·4

Table 2. *Extraction of neostigmine from a mixed sample of [^{14}C]neostigmine and 3-OH PTMA by NaTPB.*

Total (d/min)	% of total neostigmine	% of total 3-OH PTMA	Experimental % neostigmine	Experimental % 3-OH PTMA
650	87	13	86	14
300	58	42	53	47
900	10	90	10	90
38300	90	10	87	13
5600	67	33	61	39
11000	10	90	12	88

Typical results for the extraction of a mixture of the compounds from urine are shown in Table 2. The percentage of the total decompositions per minute extracted as neostigmine is calculated as the ratio of the d/min in 0·05 ml extracted from the organic layer to the total d/min contained in the same volume of urine. These figures were corrected to allow for the incomplete extraction of neostigmine (83%) and the partial extraction of 3-OH PTMA (0·7%), (see Table 1). Since neostigmine is hydrolysed to 3-OH PTMA in alkaline solutions the extraction is time-dependent and a strict routine is necessary if reproducible results are to be attained.

NaTPB also forms complexes with K^+, NH_4^+, Na^+ and other cations which may interfere with the extraction process. The extractions were made using plasma and urine obtained under mannitol diuresis and the NaTPB concentration is apparently high enough to co-extract the radio-labelled cationic compound under study together with any interfering cations present in our experimental situation. If the biological material is expected to differ in the concentration of interfering cations from that used here, re-assessment of the extraction conditions would be necessary.

Somani & his co-workers (1970) reported that 3-OH PTMA is further metabolized to a monoglucuronide. Preliminary investigations indicate that the monoglucuronide (separated from urine by paper electrophoresis) does not form a complex with NaTPB thus enabling 3-OH PTMA and the monoglucuronide to be separated by liquid cation exchange.

Department of Pharmacology and General Therapeutics, The University, P.O. Box 147, Liverpool, L69 3BX, U.K.

H. E. BARBER
G. R. BOURNE
G. A. BUCKLEY

August 25, 1972

REFERENCES

BARBER, H. E., BOURNE, G. R. & BUCKLEY, G. A. (1971). *J. Physiol., Lond.*, **219**, 4–5P.

FONNUM, F. (1968). *Biochem. Pharmac.*, **17**, 2503–2505.

FONNUM, F. (1969). *Biochem. J.*, **113**, 291–298.

SOMANI, S. M., ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1970). *Eur. J. Pharmac.*, **12**, 114–119.