J. Pharm. Pharmac., 1968, **20**, 704–708 Received February 5, 1968

The determination of bupivacaine, lignocaine and mepivacaine in human blood

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A specific method for the quantitative analysis of bupivacaine, lignocaine and mepivacaine in blood, in clinically occurring concentrations, using gas-liquid chromatography, is described. The extraction procedure yields a recovery of 97·5 (±5)%, and the chromatographic conditions allow concentrations as low as 0·04 μg/ml of local anaesthetic in 2 ml of whole blood to be measured. The method has a standard deviation of 5·4%. No interference is encountered from commonly used premedicant or general anaesthetic drugs.

THE local anaesthetics bupivacaine, lignocaine or mepivacaine may be administered in single or repeated doses to achieve local anaesthesia for surgery, or for pain relief in labour. Lignocaine is also used intravenously, in intermittent doses or by infusion, in the treatment of cardiac arrythmias. The present method was devised to allow blood concentrations of these anaesthetics to be measured after separate or simultaneous administration. The procedures developed are based in part on those described by Boyes (1967) for lignocaine and on those of Pratt, Warrington & Grego (1967) for mepivacaine.

Experimental

MATERIALS AND APPARATUS

5N sodium hydroxide. 0·1N hydrochloric acid. Analar diethyl ether, freshly distilled. Internal marker: methadone hydrochloride, 0·5 μg/ml in distilled water. Centrifuge tubes with well-fitting stoppers. 15 ml stoppered evaporating tubes with finely tapered bases (Beckett, 1966) Mechanical shaker: see-saw type mixer, 30–40 rocks/min. 10 μl Hamilton syringe.

CHROMATOGRAPHY

A Perkin Elmer F 11 gas chromatograph with a flame-ionization detector. Column: 2 metre ¼ inch o.d. glass. Solid support: Chromasorb G. 80–100 mesh. Liquid phase: 2½% silicone gum rubber, S.E. 30 (or E 301). Oven temperature: 210°. Nitrogen (carrier gas) flow rate: 70 ml/min (15 lb/inch^2). Hydrogen pressure: 20 lb/inch^2. Air pressure: 25 lb/inch^2. Packed column held under operating conditions for 24 hr, and silanized *in situ* with 2 × 5 μl hexamethyl disilazane, before use.

PROCEDURE

To 1 ml of internal marker solution in a centrifuge tube, add 2 ml of blood, 2 ml of water, 0·5 ml of 5N NaOH and 2·5 ml of ether. Stopper

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the tube, invert briskly twice, relieve the pressure, stopper and shake mechanically for 5 min. Transfer the supernatant ether layer only to a second stoppered centrifuge tube, and make three further ether extractions. Combine the four ether extracts, shake for 5 min with 5 ml 0·1N hydrochloric acid and discard the ether phase. Add 0·5 ml 5N NaOH and extract with 3 × 2·5 ml ether. Combine the ether extracts in an evaporating tube, add a glass bead and concentrate the extract in a water bath at 42°. Remove the tube from the bath and insert the stopper just as the ether vapour ceases to moisten the ground glass neck. Place the tube in crushed ice, thereby washing the inner walls with condensed ether; a volume of about 50 μl results. Evaporate further to about 20 μl by placing the tube with its base in the bath, then remove, stopper, and replace it in crushed ice (sensitivity may be increased by repeating the evaporation/condensation cycle). Mix the liquid by syringe, finally inject about 2 μl into the column of the chromatograph.

CALCULATION

Construct a calibration curve from the results obtained by adding local anaesthetics to blank anticoagulated blood to achieve concentrations found clinically (0·0625–1·0 μg/ml of each for mixtures of the anaesthetics or bupivacaine alone; 0·1–5·0 μg/ml for mepivacaine or lignocaine alone); add internal marker and proceed as described. Calculate the ratio of peak heights (PHR) of local anaesthetic to internal marker and construct a calibration curve of PHR against concentration of local anaesthetic.

The concentrations of local anaesthetics in an unknown sample are obtained by calculation of PHR and relating it to the calibration curve.

DETERMINATION OF THE PERCENTAGE RECOVERY OF THE LOCAL ANAESTHETICS AND METHADONE

The following solutions were used: (*a*) 1 μg/ml of each of the local anaesthetic bases in ether; (*b*) 2 μg/ml of methadone base in ether; (*c*) local anaesthetics added to blood equivalent to 1 μg/ml free base of each; (*d*) methadone in water equivalent to 2 μg/ml free base.

I. 2 ml of (*a*) were mixed with 1 ml of (*b*) in each of four tubes, and the solutions concentrated and subjected to gas-liquid chromatography as above.

II. 4 tubes, each containing 2 ml of (*c*) were treated as described in the general extraction procedure, but with the addition of 1 ml of (*b*) to the final ether extract before concentration.

III. 4 tubes, each containing 2 ml of (*c*) and 1 ml of (*d*) were treated as in the general extraction procedure.

Percentage recoveries of local anaesthetics were calculated by comparison of PHRS obtained in I with those in II, and that of methadone by comparison of PHRS in II and III.

SAMPLING PROCEDURE

Arterial blood samples were obtained, using a Bradley arterial catheter (Portland Plastics) and a three-way tap, flushed intermittently with heparinized saline. Venous blood samples were obtained via a Bardic angiocath, size 16, and a three-way tap, kept open with a slow infusion of physiological saline containing heparin 0·01 mg/ml. Blood samples were heparinized (about 0·2 mg/ml blood) and refrigerated pending sampling.

Results and discussion

Of the liquid phases investigated, 2½% S.E. 30 (or E 301) gave the sharpest and most readily reproducible peaks. The retention times (min) under these conditions were: lignocaine, 2·2; mepivacaine, 4·0; methadone, 5·0; bupivacaine 7·6.

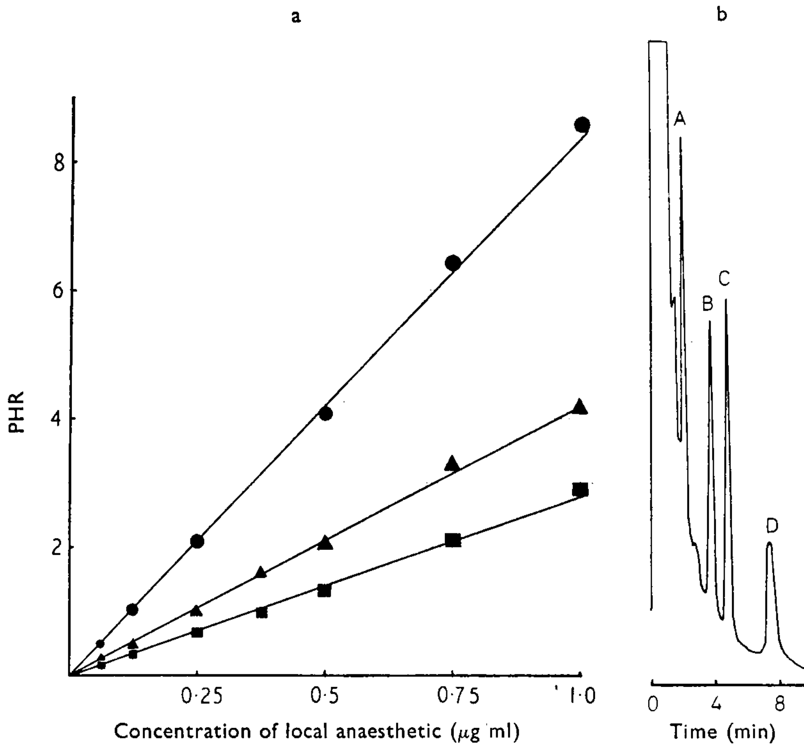

FIG. 1a. Calibration curves of bupivacaine (■), lignocaine (●) and mepivacaine (▲). PHR = Ratio, of peak height of local anaesthetic: peak height of internal marker.

b. Gas-liquid chromatogram from blood, found to contain lignocaine 0·10 μg/ml (A), mepivacaine 0·21 μg/ml (B), and bupivacaine 0·12 μg/ml (D). Methadone marker 0·5 μg (C). This figure shows lignocaine at the same sensitivity as the other drugs. The lignocaine peak height (wt for wt the greatest) is more accurately measured if the recording is made at a lower sensitivity, when the base line is flatter.

The temperature of 210° was necessary to produce an adequately sharp peak of bupivacaine at low concentrations. With a lower temperature, the peak became too blunt and wide for accurate measurement.

A linear calibration curve was obtained for each drug in whole blood (Fig. 1a). The standard deviation of eight samples containing 0·1 μg/ml of bupivacaine base (for which the analysis is least sensitive) was found to be ±5·4%. As little as 0·04 μg/ml of any of the drugs could be measured with reasonable accuracy, provided duplicate analyses were made. Consistent results were obtained with many different blood samples. Fig. 1b is a chromatogram obtained from the blood of a subject who had had 0·56 mg/kg of each local anaesthetic, intravenously, 1¾ hr before the blood sample was taken.

TABLE 1. PERCENTAGE RECOVERIES OF MIXED LOCAL ANAESTHETICS AND OF METHADONE, FROM ONE SAMPLE OF WHOLE BLOOD

	Bupivacaine		Lignocaine		Mepivacaine		Methadone
	PHR	Recovery %	PHR	Recovery %	PHR	Recovery %	Recovery %
I. Ethereal standard (mean of 4)	0·543	Represents (100%)	1·582	Represents (100%)	0·722	Represents (100%)	
II. Whole blood	0·55	101·3	1·70	107·5	0·76	105	97
	0·51	94	1·57	99	0·70	97	95
	0·483	89	1·48	93·5	0·71	98	101
	0·483	89	1·485	94	0·70	97	95
Average recovery % ± s.d.	93·26 ± 5·4		98·5 ± 6·5		99·25 ± 3·8		97 ± 2·8

The recoveries for the extraction procedure are in Table 1. The mean percentage recovery (97·5%) is much greater than that reported by Pratt & others (1967) for mepivacaine in blood (56%).

The drugs were stable in acid-citrate dextrose blood at 4° for up to one month. But, blood samples placed in heparin tubes sometimes coagulated within one week. More rapid coagulation took place if the sampling cannula was kept open by 5% dextrose instead of by physiological saline.

POSSIBLE SOURCES OF ERROR

(i) *Incomplete recovery.* Four ether extractions were found necessary. Errors were minimized by the addition of internal marker before the extraction was made.

(ii) *Contamination from the evaporating tubes.* Errors due to contamination were overcome by scrupulous washing with detergent, rinsing, chromic acid 20% and re-rinsing.

(iii) *Chromatographic interference.*

(*a*) If, during the pipetting off of the supernatant ether in the initial extractions, any of the blood phase was included, an irregular baseline was obtained.

(*b*) After injecting an extract from blood a very wide peak emerged, with a retention time of 80 min. It was therefore necessary to limit the number of samples injected in sequence to eight, with 9–10 min/sample, and then to wait for at least 80 min after the last injection to allow for eight wide peaks to emerge.

(*c*) When constructing a calibration curve from data obtained using stored acid-citrate-dextrose blood, a constant interference peak emerged, with a retention time of 14½ min. Serial injections could be timed so that this peak emerged between that of methadone and that of bupivacaine in the subsequent sample. Fresh blood did not produce this peak.

(iv) *Interference from drugs.* When stored blood is used in the preparation of a calibration curve, the local anaesthetic being measured must not have been given to the donor at the time of taking the blood. (Procaine may be used for local infiltration where necessary without interference.) No commonly encountered premedicant or other drug was found to interfere with the assay. The only other drug that emerged on the chromatogram was pethidine, but this has a retention time of 1·6 min, and therefore did not interfere.

Acknowledgements. We thank Dr. R. N. Boyes for his advice in the development of the method, Professor R. S. Stacey for his helpful criticism and support, and Messrs. Duncan, Flockhart & Evans for supplies of bupivacaine.

References

Beckett, A. H. (1966). *Dansk Tidsskr. Farm.*, **40**, 197–223.
Boyes, R. N. (1967). Ph.D. Thesis, University of London.
Pratt, E. L., Warrington, H. P. & Grego, J. (1967). *Anesthesiology*, **28**, 432–437.