Laboratory Methods

The Gas Chromatographic Determination of Mepivacaine in Blood with a Note on Other Local Anesthetics

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A gas chromatographic procedure is described for the estimation of mepivacaine after isolation from blood. Concentrations of 0.05 μg./ml. can be measured. The method has a relative standard deviation of 10 per cent over the range of 1.0 to 5.0 μg./ml. of blood. Reported is the mepivacaine level in 2 ml. samples of blood taken after low level anesthetic administration. Gas chromatographic methods are outlined which are applicable to and selective for several common anesthetic substances. Chromatograms are presented for known metabolic products of mepivacaine.

AFTER isolation from whole blood mepivacaine (Carbocaine) (*d,l* 1-methyl-2',6'-pipecoloxylidide hydrochloride) has been measured as a dye complex [1] and by reaction with cis-aconitic anhydride.[2] Sunshine and Fike [3] provided more selectivity in the estimation of mepivacaine by the two fold application of thin-layer chromatography and the "methyl orange" procedure. Direct measurement of mepivacaine by gas chromatography has been reported [4] and a gas chromatographic approach, applied to extracts from whole blood, is described here. The procedure is based, in part, on that reported by Beckett *et al.*[5] for the measurement of lidocaine (Xylocaine). This approach, applied to mepivacaine, permits quantitative measurement at levels as low as 0.05 μg. per ml. blood (fig. 1). It also makes possible qualitative identification of several other anesthetic substances as well as the identification of mepivacaine and its known metabolites.[6] Over the range of 1.0 to 5 μg. mepivacaine per ml. of whole blood, the method responds with a relative standard deviation of 10 per cent.

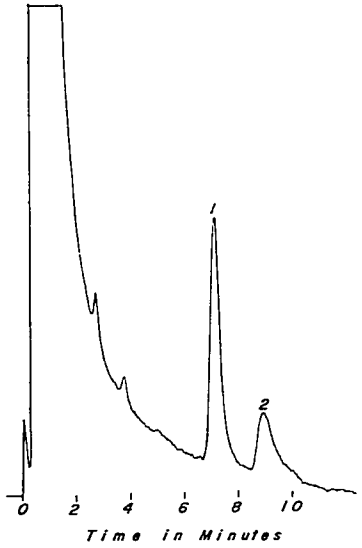

FIG. 1. The chromatogram which results after 3 μl. of a 30 μl. volume of chloroform-methanol solution containing 1.0 μg. 5 α-androstane (peak 1) and 0.1 μg. mepivacaine (peak 2) is introduced into instrument injection port.

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Methods

Principle. Whole blood is deproteinized with trichloroacetic acid. Mepivacaine base, extracted into ether, is combined with reference standard 5 α-androstane. The mixture is introduced into a gas chromatograph and the separated components are measured using a flame ionization detector.

Reagents and Apparatus

(1) Diethylether (Mallinckrodt Analytical Reagent or the equivalent)
(2) Chloroform (Mallinckrodt Analytical Reagent or the equivalent)
(3) 25 per cent trichloroacetic acid (TCA)
(4) 5 *N* sodium hydroxide, aqueous
(5) Standard Solutions
  (a) 5 α-Androstane: 25 μg./ml. in chloroform.
  (b) Mepivacaine base: 25 μg./ml. in chloroform.
(6) 15 ml. glass stoppered centrifuge tubes *invariably* cleaned as follows: Soak for 2 to 3 hours in a mixture of equal volumes of concentrated HCl and methanol. Before use, rinse four times with high quality distilled water, once with C. P. methanol, and allow to drain until dry.
(7) GLC Instrumentation. Use the following or equivalent: F and M Model 400 equipped with a flame ionization detector. The "U" shaped column, constructed of glass, has an outside diameter of 6 mm. and a length of 6 feet. The column filling consists of 3.8 per cent silicone rubber, SE-30, on Diatoport S 80–100 mesh.*

Gas Chromatographic Operating Conditions. Carrier gas (helium) flow: 75 ml. per minute at 40 psig. Instrument temperature controls: column, 200° C. and injection port, 275° C.

Procedure. Transfer 2 ml. whole blood to a 15 ml. glass stoppered centrifuge tube. Add 3 ml. distilled water to tube, followed by 1 ml. trichloroacetic acid reagent. Stopper and shake tube vigorously immediately on the addition of TCA reagent. Centrifuge the tube and its contents at high speed for about 15 minutes. Transfer the clear aqueous phase to a second 15 ml. centrifuge tube, using the all glass transfer tube illustrated in figure 2. Thoroughly mix the residue in the bottom of the initial centrifuge tube with 2 ml. distilled water, with the aid of a glass rod. Centrifuge the suspension and combine the resultant clear aqueous phase with the initial supernatant extract. Extract the combined aqueous acid extracts with three 2.5 ml. volumes of ether. Shake the stoppered tube vigorously each time, centrifuge at high speed, and finally aspirate and discard the ether phase. Add 0.5 ml. 5 *N* sodium hydroxide to aqueous phase, then 5 ml. ether. Stopper tube, shake contents vigorously and centrifuge at high speed for several minutes. Draw off the ether phase, using apparatus shown in figure 2, and collect in a dry 15 ml. centrifuge tube. Repeat extraction of aqueous alkaline phase with two additional 2.5 ml. volumes of ether. After centrifuging, in each instance, combine ether with initial extract. Evaporate ether to a volume of 3 to 4 ml. with a displacing current of nitrogen. *Do not permit complete removal of ether.* Remove any water separated from ether, with a 50 μl. syringe. Dilute 1.0 ml. of the 5 α-androstane internal standard to 10.0 ml., with chloroform. Add to the sample 1 ml. of this dilution, equivalent to 2.5 μg. internal

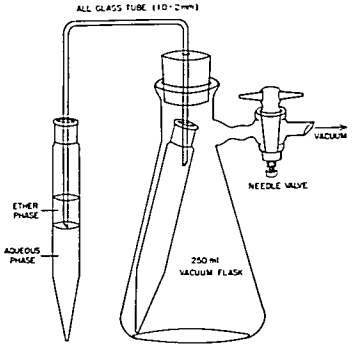

FIG. 2. Aspirating device.

* F and M Scientific Corporation, Avondale, Pennsylvania.

TABLE 1. Absolute Recovery Mepivacaine (Micrograms)

	Distilled Water 2 ml.		Whole Blood 2 ml.	
	Theory	Found	Theory	Found
	2.0	1.8	2.0	1.2
			2.0	1.2
	4.0	4.4	4.0	2.1
			4.0	2.5
	6.0	5.2	6.0	3.3
			6.0	3.3
	8.0	7.1	8.0	4.0
			8.0	5.0
	10.0	8.3	10.0	4.9
			10.0	5.4
Avg. recovery	91%		56%	
Rel. S.D.	9%		10%	

standard. Mix sample thoroughly with a glass stirring rod and evaporate to a final volume of about 30 μl. with a displacing current of nitrogen. Introduce 3 μl. of the concentrated sample containing the internal standard, into the instrument injection port, using a 10 μl. syringe.

Calibration of Peak Area. Combine 1 ml. of the 5 α-androstane internal standard solution and 1 ml. of the mepivacaine base standard solution and dilute to 10 ml. with chloroform. Transfer 1 ml. of the combined standard dilution to a clean, dry 15 ml. centrifuge tube and concentrate to a volume of about 30 μl. with a displacing current of nitrogen. Introduce 3 μl. of the concentrated standard solution into the instrument injection port, using a 10 μl. syringe.

Conversion of Detector Response to Weight Ratio. The ratio of mepivacaine base to 5 α-androstane is proportional to the ratio of their corresponding peak areas relating instrument detector response. The factor for conversion of detector response to weight ratio is obtained from the expression: F = [(μg. mepivacaine base/μg. 5 α-androstane) × (peak area 5 α-androstane)]/[peak area mepivacaine base]. Mepivacaine HCl in the sample is calculated from the expression: μg. mepivacaine HCl = [(F × μg. 5 α-androstane × peak area mepivacaine) × (1.14/0.56)†]/[peak area 5 α-androstane].

Results and Discussion

When mepivacaine is carried through the procedure described, in the absence of blood an average absolute recovery of 91 per cent is achieved. The introduction of mepivacaine

† M. W. mepivacaine HCl/M. W. mepivacaine base = 1.14; mepivacaine recovery from whole blood is 56 per cent because of systemic partitioning between solvents.

TABLE 2. Post Administration Blood Levels of Mepivacaine Hydrochloride

Patient	1	2	3	3
Body wt. (kg.)	63.5	57.4	68.0	68.0
Sex	Male	Female	Male	Male
Mepivacaine type	2% with vasoconstrictor*	3% without vasoconstrictor	2% with vasoconstrictor*	3% without vasoconstrictor
Vol. injected (ml.)	2.3	1.7	1.8	1.2
Injection site	Area of mandible	Left upper arm	Area of mandible	Area of mandible
Time after administration	Mepivacaine in Blood (μg./ml.)			
15 minutes	0.20	0.26	0.25	0.44
30 minutes	0.25	0.33	0.32	0.53
60 minutes	0.28	0.35	0.40	0.45
120 minutes	0.20	0.27	0.30	0.41
240 minutes	0.10	0.20	0.21	0.24

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TABLE 3. Storage of Mepivacaine: 10 μg./ml. in Oxalated Whole Blood

Storage Time Hours	Mepivacaine HCl Found	
	μg./ml.	% Recovery
0	10.5	105
1.5	12.3	123
5.3	10.2	102
27.5	10.0	100
95.0	11.3	113

into samples of whole blood, followed by application of the procedure, results in an average absolute recovery of 56 per cent. Analytical data obtained in the evaluation of these two types of system are presented in table 1. The differences in recovery of mepivacaine are related to the deproteinization process. Mepivacaine partitions between the components of the aqueous acid phase and the components of the solid phase which results on addition of trichloracetic acid. The relative standard deviation noted in table 1 was ob-

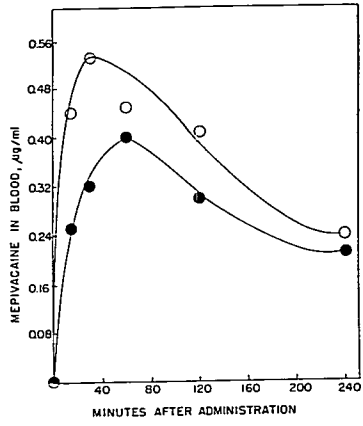

FIG. 3. Blood levels of mepivacaine measured after administration of 36 mg. drug to same patient on different days. ●——● mepivacaine and vasoconstrictor; ○——○ mepivacaine and no vasoconstrictor.

TABLE 4. Retention Times of Various Anesthetics Relative to 5α-Androstane

Liquid Phase in Column	3.8% Silicone Gum SE 30*		3.0% Silicone XE 60†		3.0% Neo Pentyl Glycol Succinate‡		7% Silicone F 60 Plus 1% EGSP-Z§
Column Temperature, °C	200	210	200	215	200	220	200
Anesthetic Substance	Relative Retention Time						
Benzocaine	0.19		1.09		2.48		0.29
1-Butyl-2',6'-pipecoloxylidide HCl	2.26						
Lidocaine	0.63		2.59		3.28		0.76
Mepivacaine	1.22		4.84	4.09	7.44	5.81	1.66
Procaine	1.00		5.05		11.64	8.42	1.46
Propoxycaine		2.75		12.7		24.8	
Tetracaine	2.09		8.29		15.62	10.6	
Reference substance							
5α-Androstane	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5α-Androstane R.T. (min.)	6.9		1.75	1.1	1.5	0.9	10.1

* Six foot glass column with 80–100 mesh diatoport S solid phase.

† Four foot glass column with 80–100 mesh Gas Chrom Q solid phase (purchased from Applied Science Labs).

‡ Four foot glass column with 80–100 mesh Gas Chrom Q solid phase (Hi Eff 3 BP, purchased from Applied Science Labs).

§ Six foot glass column with 80–100 mesh Gas Chrom P solid phase (purchased from Applied Science Labs).

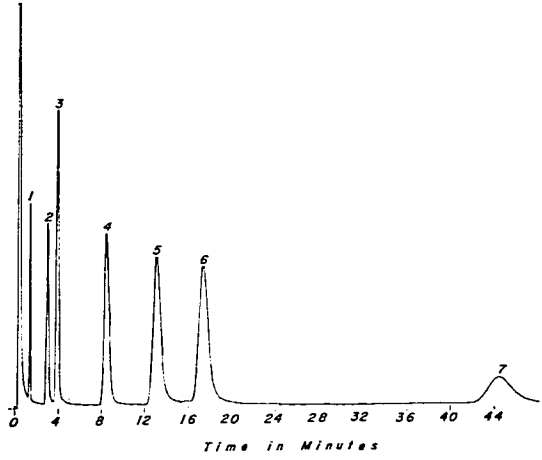

FIG. 4. Gas chromatographic separation of several anesthetic substances and 5 α-androstane. Peak 1, 5 α-androstane; peak 2, benzocaine; peak 3, Lidocaine; peak 4, mepivacaine; peak 5, procaine; peak 6, tetracaine; and peak 7, propoxycaine. Column: 3.0 per cent NeoPentyl glycol succinate with 80–100 mesh Gas Chrom Q solid phase in four ft. glass column. Column temp.: 200° C., injection port temp.: 250° C., detector temp.: 210° C.

tained from a least squares evaluation of the data. Linear rate of recovery is corroborated by a correlation coefficient of 0.98. Recovery data subsequently presented reflect the ratio of the absolute response of the sample extract corrected for partitioning, to the absolute response of reference standards.

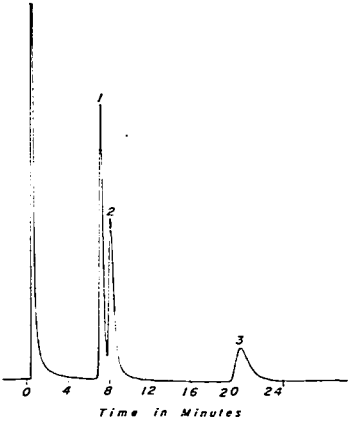

FIG. 5. Gas chromatogram of two metabolites of mepivacaine and standard 5 α-androstane. Peak 1, 5 α-androstane; peak 2, N-desmethyl mepivacaine; peak 3, 4-hydroxy mepivacaine.

The content of mepivacaine has been determined in the blood of humans receiving a relatively low dose level. Results of these determinations, obtained after gas chromatographic measurements by the procedure described herein, are presented in table 2. Each tabulated result involved the separate processing of 2 ml. of blood. The tabulated data for patient 3 are also presented in figure 3 to illustrate the influence of vasoconstrictor on the time of mepivacaine peak concentration in the blood and the quantity of anesthetic at peak concentration. Two days elapsed between the first and second administrations to patient 3, and a specimen of blood taken prior to the second administration yielded a negative finding.

Mepivacaine has been found to remain unchanged when stored under refrigeration in oxalated whole blood for a period of 95 hours. Data obtained on repeated gas chromatographic assays over this period are presented in table 3.

The gas chromatographic measurement of mepivacaine is both sensitive and discriminating. In table 4 retention times are presented for various local anesthetics relative to 5 α-androstane. From these data one may select a system best suited for the measurement of a specific anesthetic or readily select more than one system to confirm the identity of an anesthetic. A chromatogram displaying the resolution of a mixture of anesthetics is presented in figure 4.

Kristerson *et al.*,[6] have reported that metabolic products of mepivacaine, when studied in mice, occur principally in the liver. Two of the metabolites, *viz.*, *N*-desmethyl mepivacaine ‡ and 4-hydroxy mepivacaine ‡ have been subjected to the gas chromatographic procedure and the chromatograms of these substances are presented in figure 5.

Summary

A gas chromatographic procedure is described which selectively measures mepivacaine after isolation from 2 ml. volumes of whole blood. Levels of mepivacaine as low as 0.05 μg./ml. blood can be measured. The method has a relative standard deviation of 10 per cent over the range of 1.0 to 5 μg./ml. blood. A systematic partitioning loss of mepivacaine, occurring during the processing of blood, is considered in application of the procedure. Peak time and concentration of drug in blood at peak time in humans are noted after low level administration of mepivacaine. The effect of a vasoconstrictor on peak levels is demonstrated. Method selectivity for several common local anesthetics is presented as well as chromatograms of known metabolic products of mepivacaine.

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‡ Specimens supplied by Aktiebolaget Bofors, Mölndal, Sweden.

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Surgery

POSTOPERATIVE ANALGESIA Methadone and morphine when used with 25 per cent nitrous oxide in oxygen administered by a tight fitting mask and bag fitted with inspiratory and expiratory valves provides a significant improvement in pain relief over the use of opiates alone as documented by an increase in vital capacity. *(Parbrook, G. D.: Postoperative Pain Relief: Comparison of Methadone and Morphine when used Concurrently with Nitrous-Oxide Analgesia, Brit. Med. J. 2: 616 (Sept.) 1966.)*