Laboratory Methods

Gas Chromatographic Determination of Mepivacaine in Capillary Blood

J. H. Asling, M.D.,* S. M. Shnider, M.D.,† G. R. Wilkinson, Ph.D.,‡ E. L. Way, Ph.D.§

A gas-liquid chromatographic technique for the quantitative determination of mepivacaine HCl in small volumes of whole blood is described. The method is adaptable for analysis of other local anesthetics and some narcotics and antihistamines.

Transplacental transfer and fetal toxicity of local anesthetics used in obstetric anesthesia has been of interest to us.¹,² Recently, we investigated the incidence of fetal bradycardia following paracervical block anesthesia with mepivacaine hydrochloride (Carbacaine).³ We were concerned that high fetal blood levels of mepivacaine might be a causative factor. Consequently, it was necessary that both maternal and fetal blood levels of the drug be determined.

The methyl orange colorimetric procedure of Brodie and Udenfriend,⁴ modified in various ways,⁵,⁶ has been used to determine local anesthetics in body fluids. However, in our studies, where often only small blood specimens were available and other basic drugs had been administered, this method did not possess the necessary sensitivity or specificity. A number of investigators have used gas-liquid chromatography for analysis of local anesthetics in biological fluids. Beckett et al.⁷ developed a technique for the estimation of lidocaine, but 2-ml samples were required and the reported sensitivity was only 1 µg/ml. A slight modification of this procedure was reported by Fratt et al.⁸ for the determination of mepivacaine, and although it was possible to determine concentrations as low as 0.05 µg/ml, 2-ml samples were again necessary. Based in part upon these procedures, we have improved the methodology for the estimation of mepivacaine in whole blood, enabling us to determine mepivacaine using only 0.2 ml of fetal scalp blood.

Method

A Varian gas chromatograph, model 1200, equipped with a flame ionization detector, and a Varian recorder, model 20, were used for analysis. The column was stainless steel, 5 feet x ½ inch, packed with 3 per cent OV-17 coated on Gas Chrom Q, 100/120 mesh. Operating conditions were: oven temperature, 205 C; detector temperature, 275 C; injector temperature, 275 C; nitrogen flow rate, 25 ml/min; hydrogen flow rate, 25 ml/min, and air flow rate, 250 ml/min. The column was equipped with a pyrex injector insert conditioned for 24 hours at 230 C with nitrogen flow prior to use, and siliconized in situ with bistrimethylsilylacetamide.

One ml blood, 0.5 ml 5 N sodium hydroxide solution and 5 ml freshly distilled diethyl ether were added to 1 ml internal standard solution (chlorpheniramine maleate, 1 µg/ml in distilled water) in a 15-ml culture tube with a teflon-lined screw cap. The tube was shaken for 20 minutes using a tilt-action shaker, then centrifuged for five minutes. The ether phase was transferred to a clean tube containing 1 ml 1 N hydrochloric acid and again shaken. After the ether phase was discarded, the aqueous layer was made alkaline with 0.5
ml 5 N sodium hydroxide and extracted with 5 ml ether. The ether was transferred to a 10-ml centrifuge tube and concentrated to about 20 \( \mu \)l in a water bath maintained at 35 C. Using a 10-\( \mu \)l Hamilton syringe, 1 to 3 \( \mu \)l of the ether concentrate was injected into the gas chromatograph.

The concentration of mepivacaine was obtained by calculating the ratio of peak heights of mepivacaine and internal marker and relating this to a previously constructed calibration curve of mepivacaine in blood.

When 0.2-ml blood samples were analyzed, 0.8 ml physiologic saline solution was added to make one ml. When transferring ether phases, it was convenient to snap-freeze the aqueous layer in a dry ice/acetone bath. In this way the ether layer could be separated off rapidly and completely without simultaneous transfer of the aqueous layer.

To avoid chromatographic interference from glassware contaminants, it was necessary to clean all glassware scrupulously according to the following procedure. The tubes were rinsed with tap water, then allowed to stand in laboratory detergent (Alconox) for at least an hour, preferably overnight. After thorough scrubbing, the tubes were ultrasonically cleaned for 30 minutes, then rinsed successively with tap water, distilled water and 95 per cent ethanol. Periodically, and when contamination did arise, the tubes were treated overnight with chronic acid, then cleaned as above.

Results and Discussion

Figure 1 shows a typical chromatogram of mepivacaine extracted from a maternal blood sample obtained after paracervical block; the peaks are symmetrical and well resolved. A linear calibration curve was obtained over the range 0.04 to 5.0 \( \mu \)g per sample. No significant differences between recoveries from aqueous and blood samples, whether in 0.2-ml or 1-ml aliquots, were observed (fig. 2). Other drugs likely to be encountered in obstetrics, meperidine (Demerol), prilocaine (Citanest) and lidocaine (Xylocaine), did not cause interference (fig. 3).

Freezing and storage of samples up to three days did not cause significant deterioration of the drug, a finding in agreement with those of Pratt et al.\(^8\) and Reynolds and Beckett.\(^9\)

Figure 4 shows a comparative study of mepivacaine concentrations determined by our method and the methyl orange technique as modified by Daniel and Morishima.\(^6\) On the average, the methyl orange method gave results 1.15 per cent greater than those found with the gas chromatographic procedure. A logical explanation is that the buffer-solvent wash used in the methyl orange method did not completely remove interfering basic substances.

We found that back-extraction into hydrochloric acid was necessary to remove interfering compounds in blood. Use of an internal standard with extraction characteristics similar to those of mepivacaine compensated for extraction losses and permitted semiquantitative concentration of the final ether extract.
Fig. 2. Calibration curve for aqueous and 1-ml blood and 0.2-ml blood samples.

<table>
<thead>
<tr>
<th>PEAK</th>
<th>COMPOUND</th>
<th>RELATIVE RETENTION TIME</th>
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<tbody>
<tr>
<td>1</td>
<td>ETHER PEAK</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>MEPERIDINE</td>
<td>0.58</td>
</tr>
<tr>
<td>3</td>
<td>PRIOCAINE</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>LIDOCAINE</td>
<td>1.00 [10 minutes]</td>
</tr>
<tr>
<td>5</td>
<td>CHLORPHENIRAMINE</td>
<td>1.42</td>
</tr>
<tr>
<td>6</td>
<td>MEPIVACAININE</td>
<td></td>
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</tbody>
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Fig. 3. Chromatogram of related local anesthetics and meperidine.
In the procedures of both Beckett et al. and Pratt et al., the blood sample is treated with trichloroacetic acid prior to extraction. Our attempts to utilize this technique were unsuccessful, particularly when we used 0.2-ml samples. Possibly a variable loss of extractable drug during protein precipitation could account for the finding. Direct extraction of alkalized blood made recoveries relative to the aqueous standards consistent and complete, whereas Pratt et al. only obtained 58 per cent relative recovery.

After development of our assay method, Reynolds and Beckett published details of a similar procedure. The sensitivities and specificities of the two methods appear comparable. However, for routine analysis our method may have the advantage that only three extractions are necessary, rather than seven as required in the Reynolds and Beckett technique.

Although the present method yielded satisfactory estimations of mepivacaine levels in recent clinical studies, we have made preliminary attempts to increase the sensitivity of the procedure. By using a solid sampler and loading onto this a larger fraction of the ether concentrate, considerable enhancement of sensitivity can be achieved, with the added advantage that the solvent peak is removed. Alternatively, it is possible to add about 20 μl distilled carbon disulphide to the ether concentrate, then to remove the ether by a stream of air and inject 1–5 μl of CS₂ concentrate. The low detector response to this solvent permits larger samples to be chromatographed without increasing the size of the solvent peak. The blood levels found in our studies did not require the use of these modifications, but they may be used advantageously where extremely low drug levels are to be determined.

References