Laboratory Methods

The Estimation of Mepivacaine Hydrochloride in Biological Fluids

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MEPIVACAINE (Carbocaine) hydrochloride has been widely used for regional analgesia during labor and delivery. Since the compound is an unionized and relatively small molecule (m.wt. 282.8), mepivacaine may cross the placenta and reach the fetus unchanged. In order to investigate the rate of transfer of the drug from mother to fetus, its metabolism, toxcity and excretion by the newborn, it was necessary to devise a method that utilized only small quantities of fluids or tissues.

The method described here is based on the salt formation of basic organic compounds with methyl orange as described by Brodie [1] and modified by Way.[2] The drug is separated from a biological sample by extraction in ethylene dichloride at an alkaline *p*H. Organic bases with smaller molecular weight, either products of metabolism of mepivacaine or substances normally occuring in biological fluids, are removed with an aqueous solution at a suitable *p*H. The ethylene dichloride phase is shaken with methyl orange at *p*H 5 and the excess methyl orange is removed. The methyl orange salt, which dissolves in the EDC, is returned to the acid form and measured photometrically.

The procedure is a micro-modification of the method of Bromage and Robson [3] used for the measurement of lidocaine concentrations in blood.

Method

Reagents

1. *N*/10 NaOH.
2. Ethylene dichloride.
3. Dow-Corning 'Antifoam A' silicone spray.
4. Iso-amyl alcohol.
5. Phosphate buffer *p*H 6.0.
6. Saturated methyl orange sodium solution—prepared at least 24 hours in advance.
7. Saturated boric acid solution—prepared at least 24 hours in advance.
8. 1:1 mixture of saturated methyl orange and boric acid solutions freshly prepared.
9. 2 per cent sulfuric acid in absolute alcohol.

All reagents used are of Analar grade.

10. Stock standard. A solution containing 20 or 30 mug/ml is prepared by diluting the commercial solution (Carbocaine) with water.

Working Standards. Solutions containing 2–9 mug./ml. are made up daily from the stock standards and are run concurrently with each determination.

Procedure

One ml. of heparinized blood or fluid is pipetted into a 15 ml. stoppered tube containing 0.2 ml. *N*/10 NaOH, 3 ml. ethylene dichloride (EDC) and Dow-Corning antifoam A silicone spray. The tube is shaken mechanically for 20 minutes then centrifuged for 15 minutes. Mepivacaine is preferentially dissolved in the EDC. The aqueous layer is aspirated and 2 ml. of the EDC layer is pipetted into another centrifuge tube containing 1 ml. of phosphate buffer at *p*H 6.00. This is shaken for 5 minutes, then centrifuged for 10 minutes. Metabolic products of mepivacaine and other similar molecules are separated

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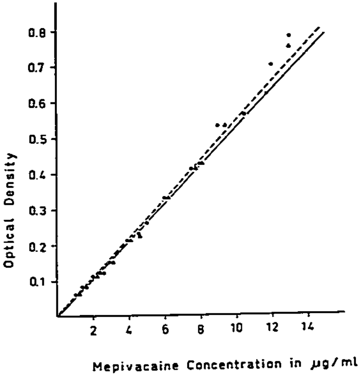

FIG. 1. Optical density of mepivacaine standards in water (●) and in blood (▲).

by this maneuver. The aqueous phase is again aspirated and mepivacaine in the EDC layer is then converted to the methyl orange salt in the following steps: 1.5 ml. of the EDC layer pipetted into a centrifuge tube containing 0.05 ml. iso-amyl alcohol, to which is added 0.2 ml. of a freshly prepared 1:1 mixture of saturated methyl orange and boric acid solutions. The tube is again shaken for 20 minutes and then centrifuged for 5 minutes. The excess methyl orange layer is carefully removed, and 1 ml. of EDC layer pipetted into a tube containing 0.2 ml. sulfric acid in absolute ethyl alcohol in order to convert the salt to its acid form. The optical density is then read at 540 millimicrons with a Coleman Junior Model spectrophotometer.

A reagent blank in which water is substituted for blood is run through the above procedure and is used for setting the instrument to zero optical density. This blank gives an optical density not exceeding 0.01 when the instrument is zeroed with ethylene dichloride.

Results and Comment

Solutions containing known quantities of mepivacaine hydrochloride were prepared with distilled water and the absorption of their methyl orange salt at 540 μ determined as described. The optical densities were then plotted against the corresponding concentrations. Known quantities of mepivacaine HCl were also added to known volumes of heparinized blood and the optical density at 540 is also plotted against the concentration in blood. Two regression lines were constructed after subtracting a corresponding blank value; they were found to be linear up to a concentration of 9 μg. mepivacaine HCl per milliliter (fig. 1). Furthermore, the two lines are almost identical and show an extraction rate greater than 95 per cent of drug from blood as compared to an aqueous solution. For concentrations higher than 9 μg./ml. a suitable dilution should be made in the final step of the determination. This is done by adding a known volume of EDC and 2 per cent sulfuric acid in absolute alcohol in the proportion 1:5.

TABLE 1. Changes in Mepivacaine Concentration in Blood Samples with Time

Sample	Day of Processing	Day of Determination	Concentration (μg./ml.)	Percentage Change
1	1	2	1.49	—
	2	2	1.42	−4.7
2	1	2	3.62	—
	2	2	3.65	+1.9
3	1	1	2.49	—
(carbocaine	1	3	2.39	−4.0
added *in*	2	3	2.35	−5.6
vitro	3	3	2.32	−6.8
	1	5	2.22	−10.9
	1	7	1.32	−47.0
	7	7	1.38	−44.5

TABLE 2. Stability of Stock Standards with Time

Concentration (μg./ml.)	Day of Analysis	Optical Density	Percentage Change
4.0	1	0.210	—
	11	0.210	−0.0
	17	0.205	−2.4
	25	0.195	−7.1
	31	0.178	−15.2
6.0	1	0.324	—
	12	0.336	+3.5
	16	0.336	+3.5
	20	0.336	+3.5
	36	0.300	−7.3
9.0	1	0.535	—
	7	0.520	−2.8
	14	0.480	−10.2

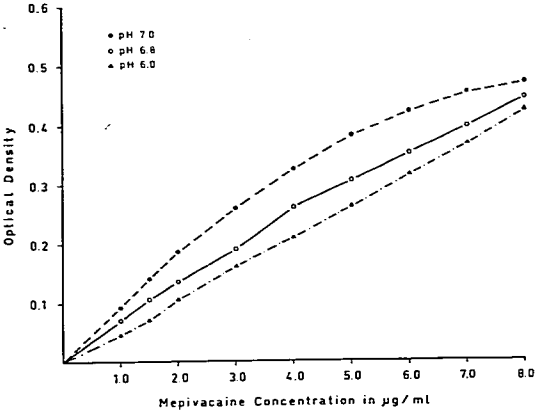

FIG. 2. Optical density of mepivacaine standards in blood using buffers of *p*H 6.0, 6.8 and 7.0.

the solution becomes cloudy if the acid is omitted.

Values in blood from patients receiving no medication varied between 0–0.40 µg./ml. when compared to a water blank; hence the method is not accurate for values in this range. In the concentration range from 1 to 9 µg./ml. the standard errors for aqueous solution and blood were 0.015 and 0.038, respectively.

When blood was stored in a refrigerator for several days prior to being processed or analyzed there was a significant loss of mepivacaine if the sample was stored for more than four days (table 1). Following one week of storage the concentrations fell almost 50 per cent.

The reading of the standards also showed some decrease with time, particularly for the more concentrated solutions (table 2). The change was considerable when standards prepared from the commercially sealed solution were kept for one month. The cause of these changes with time in both blood and aqueous solution of mepivacaine was not determined, but was probably the result of slow hydrolysis. It is therefore advisable to prepare fresh standards for each batch of determinations, or at least once every three weeks, and to process the blood samples within 4 days of collection.

Since the formation of methyl orange salt is a property of most organic bases, anesthetic agents such as meperidine (Demerol), propitocaine (Citanest) and lidocaine (Xylocaine), with structures and molecular weights similar to mepivacaine interfere with the above procedure; this method cannot therefore be used in the presence of such substances.

When ethylene dichloride was replaced with benzene as the organic solvent, both water and blank gave lower optical densities however, the optical densities of all standards were also reduced.

With the relatively small volumes used, there was no significant difference between results obtained with one or two buffer extractions. Two extractions with a phosphate buffer required an increase either in the volume of the sample or in the volume of EDC used; if more EDC was used there was a reduction in the amount of the drug present in the final step.

Maximum optical density for any concentration between 0–6 µg./ml. was obtained when a buffer of *p*H 7.0 was used. However, the standard curve was not linear the slope falling gradually as concentration increased (fig. 2), Similar results were obtained with lidocaine and propitocaine.

Summary

A method for the determination of mepivacaine hydrochloride after extraction with ethylene dichloride and removal of most of the interferring substances, is described. It can be used satisfactorily for the evaluation of concentrations of 1–9 μg./ml. using one milliliter of blood or biological fluids; suitable dilutions can be made for higher concentrations. The method is not specific and can only be used if similar drugs are absent.

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Drugs

GALLAMINE, CURARE AND HALOTHANE The hemodynamic effects of gallamine or curare administered during halothane anesthesia were investigated before surgery. The administration of gallamine increased cardiac output, heart rate, arterial pressure, and left ventricular work; decreased total peripheral resistance and mean transit time; and produced a variable response in stroke volume. Curare decreased arterial pressure and total peripheral resistance, but produced variable changes in other areas. The action of gallamine can be attributed to a cardiovagal block, myocardial stimulation, and a slight sympathetic ganglionic block. The effects of curare arise from a peripheral ganglionic block, myocardial depression, and possible histamine release. The results do not suggest any significant change in the clinical use of either of these neuromuscular blocking agents with halothane. *(Smith, N. T., and Whitcher, C. E.: Hemodynamic Effects of Gallamine and Tubocurarine Administered during Halothane Anesthesia, J.A.M.A. 199: 704 (March) 1967.)*

QUINIDINE RECURARIZATION Drug interaction is commonly observed today because of the simultaneous use of many therapeutic agents. The neuromuscular blocking effects of the cinchona alkaloids, of which quinidine is one, are well documented. This blockade seems to be related to a curariform activity at the myoneural junction, as well as a depression of muscle action potential. The failure of neostigmine to antagonize the neuromuscular blockade produced by the combination of quinidine and tubocurarine may be related to a direct effect of quinidine on muscle action potential. A clinical situation was presented in which a nondepolarizing muscle relaxant was used successfully and its action terminated as judged by clinical observation and response to peripheral nerve stimulation. The administration of quinidine at this point resulted in apnea. It is reasonable to expect that this sequence of drug administration may occur again. With awareness of the possible outcome, the physician may prevent harm to his patient. *(Way, W. L., and others: Recurarization with Quinidine, J.A.M.A. 200: 153 (April) 1967.)*