Technical Notes

Stability of Local Anesthetics After Autoclaving: Determined by Gas Chromatography

*Jordan Katz, M.D.**

THE EFFECTS of autoclaving on the stability of four local anesthetics were analyzed using gas chromatography. One per cent solutions of the hydrochloride salts of procaine (Novocain), lidocaine (Xylocaine), mepivacaine (Carbocaine), and prilocaine (Citanest) were steam-autoclaved from one to three times at 250° C. for 20–30 minutes. The local anesthetics came from their original vials or rubber stoppered ampules. Lidocaine hydrochloride contains 1 mg./ml. of methylparaben, and procaine hydrochloride methylparaben 0.5 mg./ml. and propylparaben 0.05 mg./ml., as preservatives. Stock solutions of prilocaine hydrochloride and mepivacaine hydrochloride do not contain preservatives.

Method

Five tenths milliliter of 2 *N* NaOH and 1.0 ml. of carbon disulfide (CS_2) were added to a 1.0 ml. sample of local anesthetic solution. The addition of NaOH liberates the local anesthetic base from its hydrochloride salt and makes it soluble in the organic solvent.

CS_2 was chosen as the organic solvent since the flame detector is relatively insensitive to it and thereby separations were easily made. Thorough mixing was accomplished using a Cyclo-mixer [•] for 60 seconds. Gas chromatographic analyses were done in triplicate using an Aerograph Hi-Fi Model 690 C [†] gas chromatograph.

Conditions of Analysis: Column-stainless steel, 5 feet × ⅛ inch, 2 per cent SE 30 on 100–120 mesh A/W, Chrom W DMCS; Column temperature 190° C.; injection port temperature 250° C.; hydrogen flame detector (hydrogen flow = 30 ml./minute); Carrier gas—Helium at 75 ml./minute 5 μl. injections were used.

A Leeds and Northrup [‡] script writer equipped with a Disc Model 224 integrator was used to record the chromatogram. A typical tracing is shown in figure 1. The integrated area is a representation of the concentration of the drug. By comparing integrated areas between nonautoclaved controls and autoclaved samples, changes in concentration can be determined.

Results

Results of the study are shown in table 1. The differences between autoclaved and control groups were not statistically significant as determined by the analysis of variance [1] using computer program BMD 06V (General Linear Hypothesis with Contrasts).

Discussion

Methods utilized previously for analyses of local anesthetics have involved dye complexing and quantitative colorimetric determinations.[2, 3] The complex occurs between the dye and the basic amine portion of the molecule. Unless specific extraction techniques are used, other amines, metabolites or breakdown products can not be distinguished. Figure 2 shows a chromatogram of a single injec-

* Assistant Professor, Department of Anesthesia, Stanford University School of Medicine, Palo Alto, California.

Accepted for publication May 17, 1966. Supported in part by N.I.H. Grant GM 12527.

• Clay-Adams Company, New York, New York.

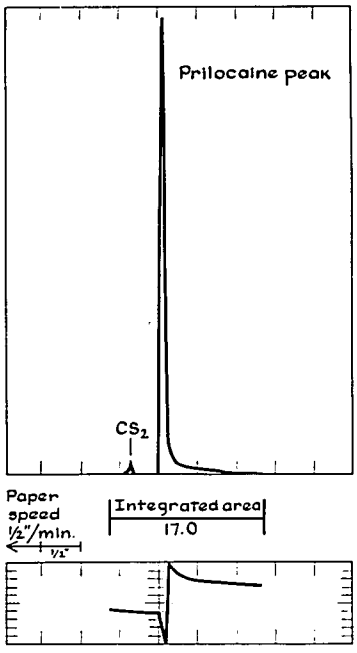

FIG. 1. Sample chromatogram of the determination of prilocaine potency.

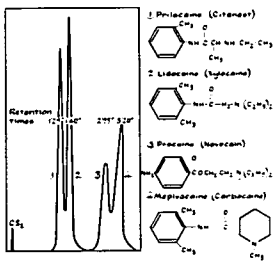

FIG. 2. Chromatogram of a mixture of four local anesthetics. Conditions of analysis: Same as in text, except that column temperature was

TABLE 1. Percentage Concentration of Autoclaved Drugs Compared with Controls*

Number of Times Autoclaved	Lidocaine (%)	Mepivacaine (%)	Procaine (%)	Prilocaine (%)
1	101.7 +2.7 −2.8	96.5 +7.4 −4.4	101.0 +13.5 −9.4	97.1 +1.8 −1.1
2	90.6 +3.8 −7.3	96.5 +6.1 −4.4	109.5 +8.8 −4.6	98.3 +3.4 −3.4
3	93.9 +3.3 −5.0	94.2 +3.2 −2.1	101.2 +12.2 −12.6	100.0 +6.2 −4.0

* Percentage Concentration

$$= \frac{\text{Integrated area of autoclaved drug}}{\text{Integrated area of control}} \times 100.$$

tion of the four local anesthetics used in this study, demonstrating that chromatographic separation, at these concentrations, is easily accomplished. Since this method can distinguish between closely related basic amines, it should be able to detect any breakdown products caused by autoclaving. There was no evidence that destruction does occur. Other columns and conditions of analysis have been used confirming the observation that the local anesthetic molecule appears stable to autoclaving.

Gas chromatographic analysis is applicable to clinical investigation. Several authors [4, 5] have described methods which are extremely sensitive and reproducible at physiological concentration of the drug. A recent report [6] describes blood levels after intravenous injections of lidocaine below 0.5 μg./ml. using gas chromatographic methods.

Conclusions

Commercially available ampules of the hydrochloride salts of procaine, lidocaine, mepivacaine and prilocaine were exposed to standard autoclaving conditions. Gas chromatographic analyses substantiated the observations that the concentration of local anesthetic is not altered by autoclaving. No breakdown products could be detected.

The author would like to express his appreci-

References

1. Schaffe, H.: The Analysis of Variance. New York, John Wiley and Co., 1959.
2. Brodie, B. B., Lief, P. A., and Poet, R.: The fate of procaine in man following its intravenous administration and methods for the estimation of procaine and diethylaminoethanol, J. Pharmacol. Exp. Ther. 94: 59, 1948.
3. Sung, C., and Truant, A. P.: The physiological disposition of lidocaine and its comparison in some respects with procaine, J. Pharmacol. Exp. Ther. 112: 432, 1954.
4. Svinhufvud, G., Ortengren, B., and Jacobsson, S. E.: The estimation of lidocaine and prilocaine in biological material by gas chromatography, Scand. J. Clin. Lab. Invest. 17: 162, 1965.
5. Beckett, A. H., Boyes, R. N., and Parker, J. B. R.: Determination of lignocaine in blood and urine in human subjects undergoing local analgesic procedures, Anaesthesia 20: 294, 1965.
6. Hargrove, R. L., Hoyle, J. R., Parker, J. B. R., Beckett, A. H., and Boyes, R. N.: Blood lignocaine levels following intravenous regional analgesia, Anaesthesia 21: 37, 1966.

PERIDURAL COMPRESSION To investigate why the same level of spinal or epidural anesthesia is achieved with less mass of local anesthetic in late pregnancies than in the nonpregnant state, radiographic measurements were made of the effect of inferior vena caval occlusion on the spread of epidural and intrathecal contrast material in dogs. The increase in pressure in the inferior vena cava caused by inflation of a cuffed catheter resulted in marked cephalad movement of epidural contrast material. A Valsalva maneuver produced the same results. Neither inferior vena caval obstruction nor Valsalva maneuvers caused comparable movement of intrathecal contrast material. (*Hipona, F. A., Yules, R., and Hehre, F. W.: Venous Encroachment on the Spinal Peridural Space Due to Experimental IVC Occlusion, Invest. Radiol. 1: 157 (March) 1966.*)

RENAL HEMODYNAMICS The combined effects of unilateral renal vasodilatation and angiotensin infusion on renal hemodynamics and sodium excretion and reabsorption were studied in anesthetized hydropenic dogs. Unilateral renal vasodilatation alone with either acetylcholine, bradykinin, or kallidin resulted in an ipsilateral increase in renal plasma flow and an ipsilateral decrease in net tubular reabsorption of sodium. Infusion of angiotensin or norepinephrine in the presence of unilateral renal vasodilatation resulted in a sustained marked increase in sodium excretion and decreased sodium reabsorption by the vasodilated kidney. These changes occurred in association with decreases in glomerular filtration rate, clearance of PAH, renal plasma flow, and noncortical plasma flow. Sodium excretion usually decreased in control nonvasodilated kidneys during the infusion of angiotensin or norepinephrine, although glomerular filtration rate was often similar in the two kidneys. The clearance of PAH, however, was always distinctly lower in the control nonvasodilated kidney. The results are consistent with the view that proper combination of two physiologically important variables, arterial pressure and renal vascular resistance, can effect large changes in the tubular reabsorption of sodium, probably through intrarenal mechanisms. Changes in these two variables may be of major importance in the regulation of sodium excretion. (*Sarley, L. E., and Friedler, R. M.: Effects of Combined Renal Vasodilatation and Pressor Agents on Renal Hemodynamics and the Tubular Reabsorption of Sodium, J. Clin. Invest. 45:*