sample patterns 8, 14, and 22, respectively, require about 1.5, 2.0, and 2.5 men per day. The assay takes a little longer than a mouse convulsion test, but is many times more accurate.

Since we were not assaying serum insulin we were able to increase volumes, standard concentrations, and I^{131}-insulin concentration to provide adequate counting quantities when low specific activity commercially available iodo-insulin was used. If high specific activity iodo-insulin is used, the sensitivity of the test could be increased by reverting to the original volumes used by Grodsky (1).

As suggested by Grodsky (1), we started using Na_2SO_3 in the salt-out step but changed to Na_2SO_4 for operation at 24°. Frictional heat arising during centrifugation led us to the use of a refrigerated centrifuge. However, since Na_2SO_4 crystallizes at 5–15°, we returned to Na_2SO_3 and 15° as our operating temperature.

We have found this test to be accurate, fast, inexpensive, and specific for natural insulin.

REFERENCES

(1) Grodsky, G. M., and Forsham, P. H., *J. Clin. Invest.*, 39, 1070(1960).
(2) Steward, G. A., *Brit. Med. Bull.*, 16, 196(1960).
(3) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, pp. 344–346.
(4) Morse, J. H., *Proc. Soc. Exptl. Biol. Med.*, 101, 722 (1959).
(5) Wilson, E. B., Jr., "An Introduction to Scientific Research," McGraw-Hill Book Co., New York, N. Y., 1952, pp. 272–274.
(6) Carpenter, F. H., and Baum, W. E., *J. Biol. Chem.*, 237, 409(1962).
(7) Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, 38, 2017(1959).
(8) Grodsky, G. M., Peng, C. T., and Forsham, P. H., *Arch. Biochem.*, 81, 264(1959).

Gas-Liquid Chromatography of Local Anesthetics and Related Compounds

By HENRY M. KOEHLER and JOHN J. HEFFERREN

Conditions for the gas chromatography of local anesthetics and related materials were investigated. Flexol on Chromosorb W and SE 30 on glass bead columns were preferred for the low and high melting compounds, respectively.

ANALYSES OF local anesthetic drugs in various dosage forms and tissue preparations have been carried out using ultraviolet, visible, and infrared spectrophotometric and titrimetric and gravimetric methods. The use of chromatographic methods for separation, identification, and quantitation of components of mixtures is well established in pharmaceutical analysis. Gas-liquid chromatography offers the advantage of rapidity combined with the sensitivity and versatility of other chromatographic techniques (1). This technique was applied to the detection and quantitation of local anesthetic bases, hydrochloride salts and degradation products. Kirk, describing the application of gas-liquid chromatography to criminalistic problems, included procaine hydrochloride among the drugs which could be chromatographed (2).

APPARATUS

The instrument used in this work was the Aerograph Hy-Fi model 600 fitted with a gold-plated hydrogen flame ionization detector and a Sargent model SR recorder with an input filter and a Disc integrator. Hydrogen, at a flow rate of 25 ml./minute, was provided for the flame ionization detector by the Aerograph hydrogen generator, model 650. Dry nitrogen was used as carrier gas at a flow rate of 30 ml./minute. Liquid phases used included Carbowax 400, Apiezon L, Ucon Polar, Flexol plasticizer 8N8, and silicone rubber SE 30. Solid supports used included 60–80 mesh firebrick, 100–120 mesh siliconized Chromosorb W, and 100–120 mesh siliconized glass beads. Five-foot columns of $^1/_8$ inch O.D. copper, aluminum, or stainless steel tubing were used.

The liquid phases were coated on the solid supports by evaporating with a Rinco rotary evaporator a slurry of the solid support and a dichloromethane solution of the liquid phase. The columns were packed with a vibrator and shaped into $2^1/_2$ inch diam. coils which were conditioned overnight in a slow stream of nitrogen at the expected operating temperature or at 200°, whichever was lower.

RESULTS AND DISCUSSION

The structural relationships of the local anesthetic agents used in this study are summarized in Table

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Samples of the local anesthetics and their component alcohols were generously furnished by their manufacturers: butacaine sulfate, benzocaine, procaine HCl, and 3-dibutylaminopropanol by Abbott Laboratories; lidocaine and *N,N*-diethylaminoacetic acid by Astra Pharmaceutical Products, Inc.; mepivacaine HCl, propoxycaine HCl and tetracaine HCl by Cook-Waite Laboratories, Inc.; diethylaminoethanol, dimethylaminoethanol, and 2,6-xylidene by Sterling Winthrop Research Institute, division of Sterling Drug; benoxinate HCl by Dorsey Laboratories, division of the Wander Co.; pyrrocaine HCl by Graham Chemical Co.; chloroprocaine HCl by Lederle Laboratories, division of American Cyanamid Co.; piperocaine HCl and *N*-(3-hydroxypropyl)-α-pipecoline by Eli Lilly and Co.; isobucaine HCl, meprylcaine HCl, 2-propylamino-2-methylpropanol, and 2-isobutylamino-2-methylpropanol by Mizzy, Inc.; butethamine HCl, metabutethamine HCl, metabutoxycaine HCl, naepaine HCl, isobutylaminoethanol, and amylaminoethanol by Novocol Chemical Manufacturing Co.; dyclonine HCl by Pitman-Moore Co., division of Dow Chemical Co.; and parethoxycaine HCl by E. R. Squibb and Sons, division of Olin Mathieson Chemical Corp.
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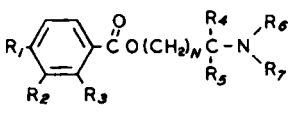
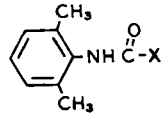
Analysis of Local Anesthetics IX. A portion of this paper was presented at the 41st general meeting of the International Association for Dental Research, Pittsburgh, Pa., March 1963.

TABLE I.—STRUCTURE OF LOCAL ANESTHETICS





	R_1	R_2	R_3	N	R_4	R_5	R_6	R_7
ESTER-TYPE LOCAL ANESTHETICS								
Procaine	NH_2	H	H	1	H	H	C_2H_5	C_2H_5
Chloroprocaine	NH_2	H	Cl	1	H	H	C_2H_5	C_2H_5
Butethamine	NH_2	H	H	1	H	H	H	$CH_2CH(CH_3)_2$
Meprylcaine	H	H	H	1	CH_3	CH_3	H	C_3H_7
Isobucaine	H	H	H	1	CH_3	CH_3	H	$CH_2CH(CH_3)_2$
Tetracaine	NHC_4H_9	H	H	1	H	H	CH_3	CH_3
Propoxycaine	NH_2	H	OC_3H_7	1	H	H	C_2H_5	C_2H_5
Naepaine	NH_2	H	H	1	H	H	H	C_5H_{11}
Parethoxycaine	OC_2H_5	H	H	1	H	H	C_2H_5	C_2H_5
Benoxinate	NH_2	OC_4H_7	H	1	H	H	C_2H_5	C_2H_5
Butacaine	NH_2	H	H	2	H	H	C_4H_7	C_4H_7
Metabutethamine	H	NH_2	H	1	H	H	H	$CH_2CH(CH_3)_2$
Metabutoxycaine	H	NH_2	OC_4H_7	1	H	H	C_2H_5	C_2H_5
Piperocaine	H	H	H	2	H	H	1-(2-methylpiperidyl)	
Benzocaine	NH_2	H	H	1	H	H_2	No aliphatic amine	
Butyl aminobenzoate	NH_2	H	H	3	H	H_2	No aliphatic amine	

	X
AMIDE-TYPE LOCAL ANESTHETICS	
Lidocaine	$CH_2N(C_2H_5)_2$
Mepivacaine	2-(1-methylpiperidyl)
Pyrrocaine	1-methylpyrrolidine

TABLE II.—RETENTION TIMES OF AMINOALCOHOLS

	Retention Time, seconds[a]		
	Column A	Column B	Column C
Ethanol	27	24	..
Aminoethanol	...	57	..
Dimethylaminoethanol	87	43	42
Diethylaminoethanol	187	43	42
Isobutylaminoethanol	246	48	50
Amylaminoethanol	489	58	68
Dibutylaminopropanol	..	66	..
2-Propylamino-2-methylpropanol	300	66	..
2-Isobutylamino-2-methylpropanol	333	48	..
N-(3-Hydroxypropyl)-α-pipecoline	108	57	..
2,6-Xylidene	...	37	..
N,N-Diethylaminoacetic acid	...	..	55

[a] Column A: 15% Flexol on 60–80 mesh firebrick, injection port temp. 185°, column temp. 170°; Column B: 15% Ucon Polar on 60–80 mesh firebrick, injection port temp. 200°, column temp. 185°; Column C: 0.1% SE 30 on 100–120 mesh glass beads, injection port temp. 150°, column temp. 100°.

I. The ester-type local anesthetics, such as procaine hydrochloride, undergo hydrolysis to the corresponding acid and aminoalcohol (3). The amide-type local anesthetics, such as lidocaine hydrochloride, are considerably more resistant to hydrolysis; however, some does occur (4).

The retention times of the aminoalcohols and other local anesthetic related compounds on three different columns are listed in Table II. The solid compounds were injected as alcoholic solutions. Normally 1–10 mcg. of the compounds gave an adequate peak. Most of these compounds contained trace amounts of the related alcohols which were easily separated. Although the Flexol column was generally the column of choice for the aminoalcohols, the over-all magnitude of the difference between the columns in Table II probably reflects the selection of the specific conditions rather than merely characteristics of the liquid phase. Identifiable peaks were observed with 1 μl. of 1% aqueous solutions of the aminoalcohols on a 15% Flexol column. Elution times generally corresponded to those seen when the pure substance was injected.

The free bases of local anesthetics are generally low melting, water insoluble solids. Usually column temperatures greater than 200° were required for the elution of these compounds (Table III). Because of relative insensitivity of the detector to carbon disulfide, this was a convenient solvent. However, reaction of the amino groups in the anesthetic molecule complicated its general use.

Local anesthetic hydrochloride salts have high melting points; for example, procaine hydrochloride melts at 153–156°. In order to minimize the injection port and column temperatures necessary to elute these high melting salts, coated glass-bead columns were used. Of the liquid phases stable at high temperatures, SE 30 proved to be the most useful. The retention times of local anesthetic salts

TABLE III.—LOCAL ANESTHETIC BASES IN CS_2[a]

	M.p., °C.	Retention Time, seconds
Mepivacaine	149–152	30
Lidocaine	66– 69	96
Procaine	60– 61	106
Pyrrocaine	82– 84	70
Tetracaine	41– 46	192

[a] Conditions: 0.1% SE 30 on 100–120 mesh glass beads, injection port temp. 265°, column temp. 210°, 0.1 μl. of 2% solution.

TABLE IV.—RETENTION TIMES OF LOCAL ANESTHETIC SALTS[a]

Local Anesthetics	M.p., °C.	Retention Time, seconds
Naepaine HCl	175–177	44
Isobucaine HCl	183–184	44
Dyclonine HCl	175–176	48
Parethoxycaine HCl	174–178	52
Lidocaine HCl	128–129	55
Piperocaine HCl	172–175	62
Pyrrocaine HCl	201–203	65
Procaine HCl	153–156	72
Mepivacaine HCl	255–262	78
Tetracaine HCl	147–150	87
Butethamine HCl	192–196	99
Chloroprocaine HCl	176–178	121
Metabutethamine HCl	116–118	133
Metabutoxycaine HCl	117–119	124
Benoxinate HCl	157–160	150
Butacaine Sulfate	100–103	192
Meprylcaine HCl	150–152	[b]
Propoxycaine HCl	148–150	[b]

[a] Conditions: injection port temp. 265°, column temp. 225°, 0.1% SE 30 on glass beads, 1 μl. 2% fresh aqueous solutions. [b] No detector response with up to 5 μl. of 2% solution at column temperatures from 100–225°.

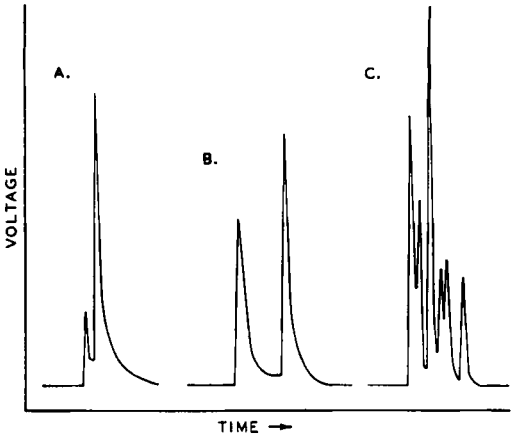


Fig. 1.—*A*, 0.1 μl. of 2% aqueous isobutylaminoethanol on 0.1% SE 30 glass-bead column; *B*, 1 μl. of 2% aqueous mepivacaine hydrochloride on 0.1% SE 30 glass-bead column; *C*, 0.5 μl. of a mixture containing 2% each of isobucaine HCl, lidocaine HCl, procaine HCl, mepivacaine HCl, and tetracaine HCl. Chart speed was 1 inch per minute.

are listed in Table IV. Columns of SE 30 on firebrick or Chromosorb W were also used; however, the results with these columns were generally less satisfactory.

Representative chromatograms of an aqueous solution of an aminoalcohol and of local anesthetic hydrochloride salts are illustrated in Fig. 1.

Several mineral acid salts of lidocaine including the hydrobromide, hydrochloride, nitrate, perchlorate, phosphate, and sulfate (5) were chromatographed on a 60–80 mesh firebrick column coated with 15% Flexol. Methanolic solutions of the base and the salts had similar retention times, suggesting that the observed peaks may be due to the free base.

Although the analyses of mixtures (6) of local anesthetics for parenteral administration, such as aqueous solutions of procaine hydrochloride with either propoxycaine (7) or tetracaine (8, 9) hydrochlorides, are possible with specific spectrophotometric procedures, gas chromatography offers a simple convenient method. Analysis of topical anesthetic mixtures, which may be aerosols, ointments, or solutions, are complicated by the frequent presence of two or more local anesthetics with quaternary ammonium compounds or other antibacterial agents in addition to pharmaceutical aids (6). The usual topical mixtures containing 2–4% lidocaine or 8–22% benzocaine with 2% tetracaine hydrochloride were readily assayed with a 0.1% SE 30 glass-bead column. A topical glycol solution of 14% benzocaine (ethyl aminobenzoate), 2% butyl aminobenzoate, and 2% tetracaine hydrochloride was assayed with the SE 30 glass-bead column. It is very difficult to quantitate each of the three local anesthetics in this mixture by any other analytical procedure.

SUMMARY

Liquid phases, solid supports, and tubing were investigated for use in the gas-liquid chromatography of aqueous and nonaqueous solutions of local anesthetics, their salts and related compounds. A Flexol on Chromosorb W column was preferred for the lower melting compounds such as the aminoalcohols, while a low load SE 30 on glass-bead column was preferred for higher melting local anesthetic bases and salts. Stainless steel tubing was preferred for the aqueous solutions of the higher melting compounds; however, no difficulties were encountered with columns prepared with copper or aluminum tubing.

The gas chromatographic procedures developed may be suitable for the determination of local anesthetics and other compounds in specific parenteral and topical local anesthetic mixtures which may not be readily assayed by the other methods.

REFERENCES

(1) Brochmann-Hanssen, E., THIS JOURNAL, 51, 1017 (1962).
(2) Parker, K. D., Fontan, C. R., and Kirk, P. L., *Anal. Chem.*, 35, 356(1963).
(3) Brodie, B. B., Lief, P. A., and Poet, R., *J. Pharmacol. Exptl. Therap.*, 94, 359(1948).
(4) Hollunger, G., *Acta Pharmacol. Toxicol.*, 17, 374(1960).
(5) Koehler, H. M., and Hefferren, J. J., THIS JOURNAL, in press.
(6) "Accepted Dental Remedies," American Dental Association, Chicago, Ill., 1963, p. 33.
(7) Feldmann, E. G., Mahler, W., and Koehler, H. M., THIS JOURNAL, 47, 676(1958).
(8) Feldmann, E. G., *ibid.*, 48, 197(1959).
(9) Pratt, E. L., *ibid.*, 46, 724(1957).