Table VI—Results (Mean ± *SD*) of Intralaboratory Collaborative Study on Chlorhexidine Digluconate ($n = 4$)

Concentration, μg/ml	Chemist A	Chemist B	Chemist C	Chemist D	Chemist E
3.5	0.057 ± 0.001	0.050 ± 0.001	0.044 ± 0.002	0.045 ± 0.002	0.036 ± 0.002
8.5	0.118 ± 0.005	0.115 ± 0.002	0.107 ± 0.002	0.109 ± 0.001	0.096 ± 0.001
16	0.236 ± 0.003	0.216 ± 0.002	0.208 ± 0.004	0.220 ± 0.003	0.215 ± 0.003
23	0.313 ± 0.005	0.299 ± 0.017	0.300 ± 0.010	0.320 ± 0.002	0.306 ± 0.001
28	0.392 ± 0.008	0.367 ± 0.008	0.376 ± 0.013	0.420 ± 0.012	0.380 ± 0.005
Correlation coefficient	0.997	0.974	0.997	0.996	0.998

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Brain-to-Blood and Saliva-to-Blood Mepivacaine Ratios in Rats

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Received June 18, 1979, from the *Department of Biological Sciences, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.* Accepted for publication August 24, 1979.

Abstract □ Mepivacaine hydrochloride, 25 and 50 mg/kg sc (with sacrifice at 15 min) produced higher ($p < 0.005$) drug levels in neonate (24–36-hr-old) rat brain and blood than in adult rat brain and blood; however, there was no significant difference in the brain-to-blood ratio of the drug between neonates and adults at either dose level. Intraarterial infusion of mepivacaine hydrochloride (20 μg/min) in adult rats resulted in measurable (GLC) mepivacaine base levels in pilocarpine-induced parotid salivary secretions collected throughout 30- and 45-min infusion periods. The saliva-to-blood ratios (±*SEM*) of mepivacaine base were 0.64 ± 0.13 after a 30-min infusion and 2.13 ± 0.48 after a 45-min infusion.

Keyphrases □ Mepivacaine—brain-to-blood and saliva-to-blood ratios, rats □ Anesthetics—mepivacaine, brain-to-blood and saliva-to-blood ratios, rats □ Models, animal—mepivacaine, brain-to-blood and saliva-to-blood ratios, rats

Several clinical studies demonstrated behavioral deficiencies and toxic manifestations in newborn infants whose mothers received local anesthesia during labor (1–8). Mepivacaine, widely used for such anesthesia, has been implicated in neonatal bradycardia, depression, apnea, convulsions, and death (1–4, 9, 10).

Because critical determinations of drug levels in the blood and tissues of healthy infants rarely are feasible, an animal model was employed to explore quantitative aspects of mepivacaine-related neural toxicity in neonates. Brain-to-blood ratios of mepivacaine base were determined for both neonate (24–36-hr-old) and adult rats after subcutaneous administration (25 and 50 mg/kg) of mepivacaine hydrochloride. Mepivacaine levels in adult rat whole blood and parotid saliva were compared to determine whether the salivary mepivacaine level could serve as an index of the blood drug concentration.

EXPERIMENTAL

Female Sprague–Dawley rats were supplied with food and water *ad libitum*. One group consisted of nonpregnant adult rats (250–300 g). Another group contained newborn rats (6–9 g) which remained with their mothers and nursed freely until drug or saline administration 24–36 hr postpartum.

Adult rats were prepared for saliva collection by anesthetization with urethan (1.2 mg/kg ip) followed by tracheotomy and cannulation of the parotid ducts according to a literature method (11). Tapered polyethylene tubing (PE 50) was inserted into each duct with the aid of a binocular dissecting microscope. Pilocarpine hydrochloride[1], 0.25 mg/ml, was infused at 0.2 ml/min into the right brachial artery to stimulate parotid salivary secretion. Mepivacaine hydrochloride[2], 1 mg/ml, was infused intraarterially (0.2 ml/min) concomitantly with the pilocarpine solution. Saliva was collected continuously throughout either a 30- or 45-min pilocarpine–mepivacaine infusion period, immediately after which 2–4 ml of blood was withdrawn *via* cardiac puncture into a heparinized vacutainer.

In another study, nonpregnant adult female rats were injected subcutaneously with 25 or 50 mg of mepivacaine hydrochloride/kg in 1.0 ml of saline; after 15 min, blood was collected *via* cardiac puncture into a heparinized vacutainer. The animals then were decapitated, and the brain was removed.

Two neonates were selected randomly from each of 15 litters and were injected subcutaneously with mepivacaine hydrochloride; one littermate received 25 mg/kg and the other received 50 mg/kg in 0.1 ml of saline. Animals were sacrificed 15 min after drug administration. Procedures for blood collection from neonates combined two techniques (12, 13). Brain tissue was removed after the spinal cord was severed at the neck.

Newborn and adult rat tissue and fluid samples also were collected from saline-injected animals, spiked with mepivacaine, and extracted for standard curves constructed each day for each tissue type. Mepiva-

[1] Aldrich Chemical Co., Milwaukee, Wis.

[2] Courtesy of Sterling-Winthrop Research Institute, Rensselaer, N.Y.

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Table I—Mepivacaine Base Concentration in Adult and Neonate Rat Whole Brain and Blood

Mepivacaine Hydrochloride[a] Dose, mg/kg sc	Brain Mepivacaine, μg/g ± SEM		Blood Mepivacaine, μg/g ± SEM		Mepivacaine Brain-to-Blood Ratio	
	Adult	Neonate	Adult	Neonate	Adult	Neonate
25	5.16 ± 0.54 $n = 18$[c]	15.50 ± 2.2[b] $n = 14$	2.61 ± 0.30	8.76 ± 1.5[b]	2.58 ± 0.39	2.01 ± 0.26
50	5.31 ± 0.52 $n = 17$	21.98 ± 2.2[b] $n = 15$	2.73 ± 0.38	11.26 ± 1.3[b]	3.84 ± 1.6	2.54 ± 0.44

[a] Animals sacrificed 15 min after mepivacaine hydrochloride administration. [b] $p < 0.005$. [c] n = number of animals.

Table II—Concentration of Mepivacaine Base in Adult Rat Parotid Saliva and Blood [a]

Infusion Time, min	n[b]	Saliva Mepivacaine, μg/g ± SEM	Blood Mepivacaine, μg/g ± SEM	Mepivacaine Saliva-to-Blood Ratio
30	17	4.79 ± 0.84	8.57 ± 1.07	0.64 ± 0.13
45	12	14.30 ± 4.64	8.10 ± 0.85	2.13 ± 0.48

[a] Mepivacaine hydrochloride was infused intraarterially at 200 μg/min. Pilocarpine hydrochloride (50 μg/min) was infused intraarterially concomitantly to stimulate parotid secretion. [b] Number of animals.

caine hydrochloride concentrations added to control samples ranged from 0.5 to 20 mg/g.

Immediately after collection, the blood, brain, and saliva samples were weighed and frozen (−15°). Because significant deterioration of mepivacaine in biological fluids stored for more than 72 hr was reported (14), extraction was performed within 3 days of tissue collection.

Published procedures were used for mepivacaine extraction from blood and saliva and for GLC analysis (14). Brain samples were homogenized with 1 ml of phosphate buffer (pH 7.4) in ground-glass tubes using a homogenizer[3]. An additional 2 ml of buffer was used to wash tissue from the homogenizing vessel into conical extraction tubes. After centrifugation at 2000 rpm for 10 min, ether was added to the supernate and the standard extraction protocol (14) was commenced.

Following evaporation of the final ether extract, the residue of each sample was reconstituted with 20 μl of chloroform. One microliter of the reconstituted sample was injected into a gas–liquid chromatograph[4] equipped with a hydrogen detector and preset to optimum conditions for detection of mepivacaine and procainamide (internal standard). The free (unbound) mepivacaine concentration (micrograms per gram of tissue) in each sample was determined *via* the method of internal standards with the use of standard curves constructed for each sample species. Relative ratios of brain-to-blood and parotid saliva-to-blood mepivacaine concentrations were calculated.

RESULTS AND DISCUSSION

Acute Mepivacaine Hydrochloride Toxicity—Dose studies (12.5–400 mg/kg) indicated that the approximate subcutaneous LD_{50} of mepivacaine hydrochloride in 24–36-hr-old neonate rats was 150 mg/kg. The subcutaneous LD_{50} of mepivacaine in rats weighing 65–75 g was reported to be 500 ± 53 mg/kg (15). None of the neonates that received 25 mg of mepivacaine hydrochloride/kg sc manifested gross toxic symptoms, but four of the 14 newborns injected with 50 mg/kg exhibited decreased spontaneous motor activity. Subcutaneous doses of 25 and 50 mg/kg produced no remarkable symptomatology in adult female rats. These subtoxic doses resulted in quantifiable mepivacaine levels in neonate and adult rat tissues.

Adult and Neonate Blood Mepivacaine Levels—Concentrations of mepivacaine base in adult and neonate rat whole brain and blood and calculated brain-to-blood ratios of mepivacaine appear in Table I. Following subcutaneous administration of 25- and 50-mg/kg mepivacaine hydrochloride doses, the mepivacaine levels were significantly higher in neonate rat brain and blood than in the corresponding adult female rat tissues. However, there was no significant difference between the brain-to-blood mepivacaine concentration ratios calculated for adults and for neonates at either of the two doses of local anesthetic agent.

Other investigators reported that a significantly greater fraction of bupivacaine, an anesthetic structurally related to mepivacaine, is bound to human maternal plasma proteins than is bound to fetal plasma proteins over a wide drug concentration range (16, 17). Although protein binding determinations were not performed, the slope of standard GLC curves (as determined by linear regression analysis) constructed with mepivacaine-spiked neonate rat brain and blood were consistently steeper than those obtained with adult rat tissue.

Acute neonatal intoxication without concomitant signs of local anesthetic intoxication in the mother was observed in humans born to mothers who received epidural or paracervical block anesthesia with mepivacaine hydrochloride during labor (1, 4, 7, 9, 18). The neurological deficiencies observed in the newborn might be related to incomplete development of the neonatal blood–brain barrier; however, this study revealed no significant differences in the brain-to-blood ratios of mepivacaine between neonate and adult rats. The study did demonstrate considerably higher levels of unbound mepivacaine in the brain tissue and blood of 24–36-hr-old rats than in these tissues of adult female rats 15 min after subcutaneous injection of equivalent body weight doses of the local anesthetic.

Mepivacaine in Adult Rat Blood and Saliva—The saliva-to-blood ratio of mepivacaine was explored to evaluate the sensitivity and reproducibility of this salivary assay as a noninvasive technique relevant to clinical monitoring of the blood level of a local anesthetic in the newborn. Intravenous infusion of mepivacaine hydrochloride (200 μg/min) in adult rats established blood and salivary drug levels well within the quantitation range by the GLC assay (14). The mepivacaine base concentrations in blood and pilocarpine-stimulated parotid saliva collected throughout 30- and 45-min intraarterial mepivacaine hydrochloride infusions and the saliva-to-blood mepivacaine ratio for each collection period are summarized in Table II.

Although the salivary drug concentration data were reasonably consistent within any collection period (*i.e.*, 30 or 45 min), the saliva-to-blood mepivacaine ratio differed considerably, depending on the infusion time and the total dose of local anesthetic. Thus, this study failed to demonstrate a consistent relationship between the mepivacaine level in these two body fluid compartments that would support saliva sampling and analysis as a means of estimating the blood drug level. The salivary secretion rate in the rat necessitated collection of a 20–30-min sample for drug extraction; a larger animal model, permitting simultaneous sampling of saliva and blood, would be advantageous to explore the relative compartmentalization of mepivacaine.

[3] Dorenmeyer.
[4] GOW-MAC model 750, Bound Brook, N.J.

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GLC Microdetermination of Indomethacin in Plasma

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Abstract □ A GLC method utilizing an electron-capture detector is described for analysis of indomethacin in blood plasma. Indomethacin is extracted with ethyl acetate from plasma buffered to pH 5.0. The ethyl acetate is evaporated to dryness, and indomethacin is derivatized to a pentafluoropropionyl ester prior to chromatography. A GLC standard is used for peak height quantitation of indomethacin. The extraction efficiency from plasma is 92 ± 3%, and as little as 1 ng of indomethacin can be quantitatively determined.

Keyphrases □ Indomethacin—GLC analysis, human plasma □ GLC—analysis, indomethacin in human plasma □ Anti-inflammatory agents—indomethacin, GLC analysis in human plasma

Indomethacin[1] is an extensively used anti-inflammatory drug with potent inhibitory action on prostaglandin synthesis. Recent studies demonstrated that the drug is also successful in the management of patent ductus arteriosus in the premature infant (1). Since complications of indomethacin therapy in the premature infant are associated with high plasma levels, a rapid and sensitive assay was needed for monitoring therapeutic plasma drug levels utilizing small samples. Several investigators recently reported sensitive analytical procedures for indomethacin in biological fluid; however, these procedures require several extractions (2, 3), lack an internal standard (4), and require an initial plasma sample of 0.5 ml (5). Therefore, an electron-capture GLC method was developed for monitoring therapeutic indomethacin levels in limited plasma samples (0.1 ml) utilizing a GLC standard for quantitation.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a ^{63}Ni-electron-capture detector was maintained with a high purity nitrogen column gas flow of 70 ml/min. The column oven temperature was 295°; the injection port and detector temperatures were maintained at 310°.

Column—A glass column, 2 m × 2 mm, was packed with 10% SP 2250 on 100–120-mesh Supelcoport[3]. The column was rinsed before packing with methanol and acetone; it was then dried and conditioned for 2 hr with a 20% solution of dimethyldichlorosilane[4] in toluene. Following silylation, the column was rinsed with acetone and dried under nitrogen before packing.

Procedures—Plasma (0.1 ml) was transferred to 1.5-ml polypropylene disposable micro test tubes[5], buffered to pH 5.0 with 0.1 ml of 0.2 *M* acetate buffer, and extracted with 1.0 ml of ethyl acetate. The ethyl acetate (0.9 ml) was transferred by disposable pipet to a dry, clean 1.5-ml micro test tube and evaporated to dryness using a vacuum centrifuge[6]. The residue was mixed with 10 μl of a mixture of 2,2,3,3,3-pentafluoro-1-propanol in pentafluoropropionic anhydride[7] (1:4, v/v) and heated to 75° for 20 min. Following derivatization, the sample was evaporated to dryness using the vacuum centrifuge; the residue was redissolved in 10 μl of ethyl acetate containing 2.5 μg of *p*-bromobenzaldehyde isonicotinoylhydrazone/ml as the internal standard.

One-microliter samples were injected into the gas chromatograph for analysis. The internal standard was prepared according to Timbrell *et al.* (6) by mixing equimolar amounts of isoniazid and *p*-bromobenzaldehyde in methanol and refluxing for 1 hr at 60°. After cooling for 24 hr at −20°, the crystalline product was filtered, washed twice with warm methanol, and recrystallized. The final product, *p*-bromobenzaldehyde isonicotinoylhydrazone, was found to be better than 98% pure by GLC and TLC.

A standard calibration curve was prepared by adding 0.1 ml of plasma to known amounts of indomethacin to give final indomethacin concentrations ranging from 0.01 to 1 μg/ml. Thirty samples, five per concentration, were analyzed to prepare the standard curve. Each sample was injected in duplicate, and the determination of the entire standard curve was replicated at once. 2-^{14}C-Indomethacin[8] was used to estimate the extraction efficiency of indomethacin from plasma by measurement of the total radioactivity in the extract.

RESULTS AND DISCUSSION

The electron-capture GLC retention times for derivatized indomethacin and the internal standard were 5.2 and 6.8 min, respectively. Chromatograms from a control human plasma and a plasma sample containing 31 ng of indomethacin, both with the internal standard, are shown in Fig. 1. Since peaks for both agents were symmetrical, quantitation was made by comparison of peak heights. Detector response and the calibration curve were linear over 0.01–1.0-μg/ml range. Blanks were prepared from plasma of drug-free subjects, and no peaks were observed that would interfere with the measurement of indomethacin or the internal standard. Plasma samples containing 5 μg/ml of salicylate or furosemide also did not interfere with indomethacin quantitation.

Linear regression analysis of the individual data points (n = 60) from the standards plotted as the ratio of the peak height of derivatized in-

[1] Indocin, Merck & Co.
[2] Varian model 3700.
[3] Supelco, Inc., Bellefonte, Pa.
[4] Applied Science Laboratories, State College, Pa.
[5] Bio-Rad Laboratories, Richmond, Calif.
[6] Savant Instruments, Hicksville, N.Y.
[7] Regis Chemical, Morton Grove, Ill.
[8] New England Nuclear, Boston, Mass.

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