Binding of Anilide-type Local Anesthetics in Human Plasma:

I. Relationships between Binding, Physicochemical Properties, and Anesthetic Activity

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The rank-order of binding in human plasma for a series of anilide-type local anesthetics, determined by ultrafiltration and gas chromatography, was: bupivacaine > mepivacaine > lidocaine > the N-dimethyl analog of lidocaine. Drugs were added to plasma *in vitro*, pH maintained at 7.4 ± 0.1 and temperature, 26 ± 2 C. Attempts to identify the plasma-binding factor proved unsuccessful. However, binding to plasma lipoproteins was not entirely ruled out owing to difficulty in dispersing corresponding Cohn fractions in buffer. Extensive plasma-binding of bupivacaine was confirmed by equilibrium-dialysis and gel-filtration experiments. Good correlation between extent of binding and distribution of the drugs between plasma and erythrocytes was found. The distribution between buffer and erythrocytes indicated that binding to erythrocytic components had the same rank-order as binding to plasma. Relationships between the binding properties, physicochemical characteristics and anesthetic activity are discussed. Durations of anesthesia, determined in isolated nerve preparations correlated with the binding characteristics. (Key words: Bupivacaine; Mepivacaine; Lidocaine; Plasma-binding; Ultrafiltration; Plasma/erythrocyte distribution; Physicochemical properties; Duration of anesthesia.)

BECAUSE MACROMOLECULES and, therefore, drugs bound to macromolecules, are assumed to cross biological membranes only with difficulty, it is generally considered that the binding of drugs in plasma can exert an important controlling effect on many of the processes involved in drug distribution, elimination, and therefore, action. Some of the confusion surrounding the implications of plasma binding of drugs has been dispelled by two timely reviews.[1, 2]

Analysis of the literature on protein binding of drugs revealed an emphasis on studies involving acidic compounds, with little information about binding of the more numerous and generally more potent basic drugs.[1] Data about the binding of local anesthetic agents are also relatively sparse. There have been reports of limited investigations of the binding of procaine (Novacaine) in rabbit and dog plasma using an ultrafiltration method[2]; binding of ester-type local anesthetics to bovine serum albumin using equilibrium dialysis[4]; binding of procaine, butethamine (Monocaine), lidocaine (Xylocaine), and mepivacaine (Carbocaine) to human serum albumin and gamma-globulin using equilibrium dialysis[5]; human serum-binding of lidocaine and prilocaine (Citanest) using ultrafiltration[6]; and human plasma binding of lidocaine using both ultrafiltration and equilibrium dialysis.[7]

In the present communication we report attempts to establish the relative plasma-binding properties of a series of anilide-type local anesthetics, including the two agents most commonly used, lidocaine and mepivacaine, and the newer, long-acting compound, bupivacaine (Marcaine). We also attempted to identify plasma components responsible for binding, and to develop the relationships between binding, physicochemical, and pharmacologic properties of the compounds.

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Methods

Plasma Binding

Ultrafiltration. At weekly intervals, 100-ml samples of blood from a 31-year-old man (Subject 1) were collected in heparinized tubes. All samples were taken between 9 and 10:00 AM. Routine biochemical and hematologic tests indicated no abnormalities. Plasma was separated from the cells by centrifuging at 2,000 rpm for six minutes and concentrations of 0.04–20 μg base equiv/ml lidocaine hydrochloride, (±)-mepivacaine hydrochloride, (±)-bupivacaine hydrochloride, and W-36017 hydrochloride (the N-dimethyl analog of lidocaine) were prepared in 5.0-ml amounts. Dilution of plasma was minimized by adding the drugs in small volumes (2–50 μl) of aqueous solutions using microliter syringes.§ We allowed for dilution factors in subsequent calculations. Within 30 minutes of withdrawal of the blood, the drug-loaded plasma was placed in 10-ml conical flasks, previously treated with a siliconizing fluid,¶ and a 5% carbon dioxide–95% oxygen mixture was passed in to stabilize the *p*H at 7.4 ± 0.1. The tightly stoppered flasks were then left at room temperature (26 ± 2 C) for 30 minutes, with periodic shaking.

Lengths of dialysis tubing** which had been soaked in distilled water for an hour were carefully dried with tissue paper. The plasma samples were then transferred into the tubing, which was looped inside 15-ml glass centrifuge tubes such that the ends could be fastened by the stopper. The bottom of the tubing was positioned to be clear of the level ultimately reached by the ultrafiltrate. The carbon dioxide–oxygen mixture was passed into the tubes before application of the stoppers, which subsequently were sealed with waxed paper.†† The tubes were then centrifuged‡‡ at 700 × *g* for two hours. The resultant 1-ml ultrafiltrates were analyzed for drug content by a method based on gas chromatography,[8] which has a coefficient of variation of ±4% at the 0.1 μg/ml level. For reasons outlined previously,[8] 1 ml of "blank" blood was added to each sample prior to analysis. At low drug concentrations, duplicate determinations of binding were carried out to increase confidence in the analysis. The extent of plasma-binding at each concentration was calculated from the concentrations of drug in plasma and ultrafiltrate. The latter was determined to be free of protein. Controls containing no protein and consisting of solutions of the drugs in isotonic Sørensen's phosphate buffer, *p*H 7.4, were also subjected to ultrafiltration.

The following additional experiments were performed, using the above procedure. The concentration of drug used was 5 μg/ml in each case.

1. Intra- and intersubject variation in plasma binding of each drug was investigated by repeating ultrafiltration with plasma from subject 1 obtained on different occasions, and with plasma from six other donors. Male and female subjects, none of whom were on drug therapy, were used. For subject 2, binding was determined in plasma analyzed after addition of local anesthetics to whole blood.

2. Plasma-binding characteristics of the (+)- and (−)-isomers of mepivacaine and bupivacaine were compared with those of the racemic forms. A pool of plasma from six donors was used and the experiment carried out in duplicate.

3. Binding of bupivacaine in "platelet-poor" plasma, prepared by centrifuging plasma at 3,000 rpm for 30 minutes, was compared with binding in "normal" plasma. A pool of plasma from six donors was used and the experiment carried out in duplicate.

4. Physiologic concentrations[9] of commercial Cohn fractions of human plasma§§ in both isotonic Sørensen's phosphate buffer (*p*H 7.4) and 0.9% sodium chloride solution were prepared. The respective fractions and the approximate percentage contents of their major plasma protein components were: fraction I 60% fibrinogen; II, 100% gamma-globulin; III 76% beta-globulin (lipoprotein); IV-1, 90% alpha-globulin (lipoprotein); IV-4, 84%

§ Hamilton Co., Whittier, California.
¶ Desicote-Beckman Instruments, Inc., Fullerton, California.
** Seamless, regenerated cellulose, viscose process, flat width 7/16 in, Union Carbide Corporation.
†† Parafilm "M," American Can Co., Neenah, Wisconsin.
‡‡ Sorvall Automatic SS-3, Ivan Sorvall, Inc., Norwalk, Connecticut.
§§ Nutritional Biochemicals Corp., Cleveland, Ohio.

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alpha- and beta-globulins; V, 95% albumin. Binding of lidocaine and bupivacaine to the separate fractions and in a synthetic plasma made by mixing all the fractions was investigated.

Equilibrium Dialysis. Sufficient bupivacaine was added to pooled human plasma, freshly prepared plasma ultrafiltrate, and freshly prepared ultrafiltrate containing 4% crystalline human albumin§§ to produce 5 μg/ml solutions. Five-milliliter amounts of these solutions were placed within lengths of cellulose dialysis tubing,¶¶ tightly tied at both ends, and placed in glass vials containing 5.0 ml of plasma ultrafiltrate. The vials were closed with screw-caps and attached to a vertically-rotating wheel located in a cold room at 4 C. Dialysis was continued for 24 hours. Drug-loaded plasma (5.0 ml) was dialyzed against drug-free albumin solution (5.0 ml) in a further experiment. Any changes in volumes of the internal and external dialysis phases were noted at the end of the experiments. The extent of binding was estimated after analysis of the drug in the two phases after equilibration.

Gel Filtration. Sephadex G-25 (medium grade)*** was allowed to swell overnight in isotonic Sǿrensen's phosphate buffer, *p*H 7.4, and packed into a 10-ml serologic pipet,††† plugged with glass wool, to produce a column 120 × 5 mm. This was then equilibrated for at least four hours with buffer at room temperature supplied at approximately 0.1 ml/minute from a siphon arrangement. Plasma (1.0 ml containing 5 μg bupivacaine) was applied to the top of the column and 10-drop fractions (approximately 0.6 ml) were collected during subsequent elution with 9–10 ml of buffer. Protein concentrations were determined spectrophotometrically‡‡‡ in 0.1-ml samples of the fractions by measuring the height of the maximum at 280 mμ. Bupivacaine in the remaining 0.5 ml was determined by gas chromatography.[8] Corrections were made for variations of drop size during the runs. As a control, 1.0 ml of buffer containing 5.0 μg of bupivacaine was treated similarly.

Distribution in Blood

Plasma/Erythrocyte Distribution. Blood samples from subjects 1 and 2 were collected in heparinized tubes and hematocrits were determined in triplicate using a microtechnique. Within 30 minutes of collection, concentrations of 2, 5, 10, and 20 μg base equiv/ml of lidocaine HCl, (±)-mepivacaine HCl, and (±)-bupivacaine HCl were prepared in 5.0-ml samples of the blood placed in siliconized 10-ml conical flasks. Dilution of the blood was minimized by adding the drugs in small volume (5–50 μl). The *p*H of the samples was stabilized at 7.4 as described above and the tightly stoppered flasks were left at room temperature for 30 minutes, with periodic shaking. The samples were then divided, half being centrifuged to obtain the plasma. Drug concentrations in samples of whole blood (C_t) and plasma (C_p) were determined by gas chromatography.[8] Concentrations of unbound drug (C_f) in plasma samples from subject 2 were determined directly by ultrafiltration and those in plasma samples from subject 1 by extrapolation from binding data previously determined. Apparent concentrations of the drugs in erythrocyte water (C_{ew}) were calculated using the equation:

$$C_{ew} = 1.40 \times \frac{C_t \cdot 100 - C_p \cdot (100 - H)}{H} \quad (1)$$

where H = hematocrit. The factor 1.40 represents the reciprocal of the fractional water volume of erythrocytes.[10] Drug concentration in plasma water (C_{pw}) was calculated by multiplying C_f by the factor 1.06.[15] A plot of C_{pw} versus C_{ew} was constructed for each drug.

Buffer/Erythrocyte Distribution. Erythrocytes from the same subjects were washed and suspended in isotonic Sǿrensen's phosphate buffer, *p*H 7.4. Hematocrits were adjusted to 40 per cent. Concentrations of 1 and 5 μg base equiv/ml (and in one subject, 10 μg/ml also) of the three local anesthetics were prepared in 5.0-ml samples of the buffer–erythrocyte solutions. After standing and shaking, the "whole-blood" (C_t') and buffer supernatant (C_b) concentrations of the drugs were determined as

¶¶ Union Carbide Corp. (flat width 15/16 in.).
*** Pharmacia Fine Chemicals Inc., Piscataway, New Jersey.
††† Kimble Products, Toledo, Ohio.
‡‡‡ Beckman DK-1, Beckman Instruments, Inc., Fullerton, California.

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described above. Apparent drug concentrations in erythrocyte water (C_{ew}) were determined using equation 1, but substituting C_b for C_p. The relationship between C_b and C_{ew} was compared with that between C_{pw} and C_{ew} determined previously. Also, the percentage of each drug associated with the erythrocytes (%E) was plotted as a function of C_t'.

Determination of Partition Coefficients

The "rocking method" of Reese *et al.*[11] was used to determine partition coefficients of (±)-bupivacaine, (±)-mepivacaine, lidocaine, and W-36017 for the system n-heptane/isotonic phosphate buffer, *p*H 7.4. Equal volumes (10.0 ml) of all phases were employed, the drugs being added as hydrochloride salts to the aqueous phase. The tubes containing the partitioning system were rocked slowly at room temperature (25 ± 2 C) for 20 hours on a tilt shaker. Final concentrations (C_{final}) of the drugs in the buffer phase were determined by gas chromatography.[8] Experiments were carried out in triplicate for each drug at two initial concentration levels ($C_{initial}$, *viz.*, 20 and 100 μg/ml buffer). Tubes containing only the drugs in buffer solution were included as controls.

Partition coefficients (P) were calculated using the equation:

$$P = \frac{(C_{initial} - C_{final}) \text{ in buffer}}{C_{final} \text{ in buffer}} \quad (2)$$

Equation 3 was used to calculate the partition coefficients (P^1) of the nonionized forms of the drugs, assuming that only these forms passed into the organic phase[12]:

$$P' = P(1 + 10^{pK_a - pH}) \quad (3)$$

Buccal Absorption Test (B.A.T.)

The method of Beckett and Triggs[13] was employed with modifications suggested by Beckett and Moffat.[14] Three subjects were tested using McIlvane's citric acid-phosphate buffer, *p*H 7.4, and drug concentrations of 20 μg base equiv/ml. Subjects received solutions of lidocaine, (±)-mepivacaine, (±)-bupivacaine, and W-36017 hydrochloride on different occasions according to a Latin square design. The test was also carried out with a solution containing both lidocaine and bupivacaine.

Table 1. Binding of Anilide-type Local Anesthetics in Human Plasma as a Function of Drug Concentration

	Total Drug Concentration in Plasma (μg/ml)	Per Cent Binding			
		Bupivacaine	Mepivacaine	Lidocaine	W-36017
Subject 1	0.4	96.5	84.3	75.0	39.0
	1.0	96.2	82.3	70.6	37.4
	2.0	95.6	77.5	64.3	35.5
	5.0	87.7	67.8	58.4	30.7
	10.0	76.5	52.8	46.0	23.4
	20.0	61.5	38.3	28.2	17.6
Subject 2*	1.6	—	—	66.8	—
	2.9	—	74.2	—	—
	3.6	90.6	—	—	—
	6.6	—	63.3	—	—
	6.9	—	—	53.0	—
	7.7	84.0	—	—	—
	11.7	—	49.1	—	—
	12.6	71.1	—	—	—
	12.7	—	—	40.4	—
	23.3	—	31.5	—	—

* Binding determined in plasma analyzed after addition of drugs to whole blood.

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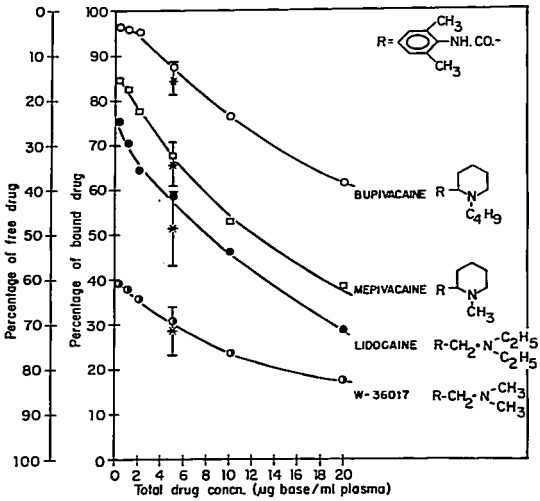

FIG. 1. Concentration dependence of human plasma-binding of anilide-type local anesthetics, determined *in vitro* by ultrafiltration. (Data for plasma from subject 1. Mean percentage binding of each drug ± 1 SD in plasma from six subjects at the 5 μg/ml level is also indicated.)

Results

PLASMA BINDING

Ultrafiltration. The results (table 1) indicate that for all of the drugs plasma-binding is inversely related to total drug concentration. This concentration dependence is shown graphically in figure 1. The rank-order of binding was: bupivacaine > mepivacaine > lidocaine > W-36017.

Results of the buffer-control experiments indicated that no significant quantities of the drugs adhered to the dialysis membranes over the concentration range studied. Preliminary experiments demonstrated that the extent of binding was independent of time, at least over the period five minutes to four hours, and that the use of small volumes of drug solutions in preparing plasma–drug concentrations was as accurate as using 1-ml volumes of more dilute drug solutions. Heparin did not affect binding, in that similar results were obtained using serum (no anticoagulant present) and using plasma. Furthermore, results of ultrafiltration of drugs added to heparinized buffer were identical to those obtained with ordinary buffer controls. The concentration of plasma constituents as ultrafiltration proceeded did not necessitate a correction in the calculation of binding; also, the plasma volume was not corrected for an estimated volume occupied by the plasma proteins.§§§.[15, 16] For technical reasons it was not possible to study binding at 37 C; temperature recorded in the centrifuge after ultrafiltration was approximately 2 C above room temperature.

When the nature and concentration of the macromolecule responsible for binding is not known, Rosenthal[17] has advocated the use of a Scatchard-type plot, base on equation 4, to allow calculation of binding constants:

$$\frac{D_b}{D_f} = nkP_t - kD_b \qquad (4)$$

where

D_b = molar concentration of bound drug in plasma

§§§ In the case of cationic compounds, concentration in an ultrafiltrate should essentially equal free concentration in whole plasma.[15] The local anesthetics will be > 50% ionized at plasma *p*H; therefore, we have assumed this relationship arbitrarily.

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TABLE 2. Plasma-binding characteristics of Anilide-type Local Anesthetics, Calculated from Ultrafiltration Data

Compound	Affinity Constant $k(mM^{-1} \pm 1\ SD)$	nP_t (mM)	Correlation Coefficient for D_b/D_f vs. D_b
W-36017	30.5 ± 2.8	0.022	0.983
Lidocaine	101.4 ± 13.9	0.027	0.965
Mepivacaine	135.2 ± 36.1	0.036	0.882
Bupivacaine	a 653.9 ± 123.8	0.038	0.935
	b* 979.9 ± 87.3	0.028	0.988

* Neglecting data point equivalent to 20.0 μg/ml total plasma drug concentration.

D_f = molar concentration of unbound drug in plasma
P_t = total molar concentration of macromolecule
k = affinity constant (mM^{-1})
n = number of binding sites

A plot of D_b/D_f as a function of D_b is independent of macromolecular concentrations and allows estimation of the parameters nP_t and k from intercepts and slope. Values for these variables and the correlation coefficient for the line obtained by plotting ultrafiltration data for each drug in this manner are shown in table 2. The similarity of the figures for nP_t suggests that the drugs bind to a common site. Although reasonable correlation coefficients were obtained, the plots did show a trend towards convex-decreasing curvature, indicating that more than one type of macromolecule or more than one class of binding site on a single type of macromolecule may be involved in the binding. Alternately, increasing the concentrations of drug may cause exposure to greater numbers of identical binding sites. A degree of curvature was also apparent when double-reciprocal plots ($1/D_b$ vs. $1/D_f$) were constructed.

The binding characteristics of all the drugs in the plasma of subject 2 were reproducible and at the 5 μg/ml level were consistently greater for each drug than the mean percentage binding values determined in plasmas from six subjects. No significant stereoselectivity was apparent in the binding of mepivacaine or bupivacaine. Also, no significant difference between the binding of bupivacaine in plasma and that in "platelet-poor" plasma was observed (table 3).

No appreciable binding of lidocaine or bupivacaine to any of the Cohn plasma protein fractions, either separately or in admixture, was observed. Results with buffer and saline solutions were similar. Unfortunately, binding to plasma lipoproteins could not be measured conclusively owing to the difficulty in dispersing corresponding Cohn fractions (III and IV-1) in aqueous solution.

Equilibrium Dialysis. The volumes of the solutions within and outside the dialysis bags were chosen to duplicate those used by Shnider and Way.[7] Dialysis was not carried out at 37 C as in their experiments, but rather at 4 C, in order to minimize deterioration and/or bacterial contamination of the plasma which was apparent at the higher temperature.

In the control experiment bupivacaine was found in equal concentrations within and outside the dialysis bag after 24 hours. Extensive plasma-binding of bupivacaine was observed when plasma was dialyzed against both plasma ultrafiltrate and albumin solution. No appreciable binding in albumin was suggested when the latter was dialyzed against ultrafiltrate. The conditions employed did not allow accurate quantitative estimation of plasma binding, as the osmotic pressure of the protein caused fluid to be transferred from the outside of the bag to the inside, thus diluting the plasma.

Gel Filtration. Figure 2 shows that free bupivacaine was adequately resolved from plasma proteins with the system used. Addition of bupivacaine in plasma caused the drug to elute in two main peaks, the larger associated with the plasma protein peak and the smaller associated with the free-drug peak. Resolution of the latter peaks was not complete, however, indicating that some dissociation of the drug–macromolecule complex occurred during elution with buffer. From the results we estimated that approximately 65% of the bupivacaine was associated with the plasma-protein peak. Recoveries of bupivacaine, after addition to the column in both plasma and buffer solutions, were essentially complete.

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TABLE 3. Human Plasma-binding of Anilide-type Local Anesthetic Agents, Determined by Ultrafiltration

	Per Cent Binding (Total Concentration of Each Drug = 5 μg/ml)			
	Bupivacaine	Mepivacaine	Lidocaine	W-36017
Intrasubject variation: subject I, two determinations	87.7; 90.0	65.0; 67.8	55.6; 58.4	30.7; 29.0
Intersubject variation: mean ± 1 SD, six subjects*	84.7 ± 3.4	65.9 ± 4.9	51.2 ± 8.2	28.6 ± 5.4
Stereoselectivity:				
(±)-isomer	90.4; 85.2	64.6; 65.0	—	—
(+)-isomer	91.1; 86.4	68.2; 65.9	—	—
(−)-isomer	89.0; 81.1	62.6; 61.3	—	—
(plasma pools from six subjects)				
Platelets:				
normal plasma	82.0; 84.1	—	—	—
"platelet-poor" plasma	85.1; 86.6	—	—	—
(plasma pools from six subjects)				

* Differences between binding capacities of the drugs were significant at $P < 0.001$ (Student's t test).

DISTRIBUTION IN BLOOD

The results of the *in vitro* studies of distribution of the local anesthetics between plasma and erythrocytes and between buffer and erythrocytes are shown in tables 4 and 5. Preliminary experiments indicated that distribution was independent of time from five minutes to four hours.

Figure 3 shows the relationships between the concentrations of free drug in plasma water and in buffer supernatant and the apparent concentration of drug in erythrocyte water for each of the local anesthetics. The data for plasma and buffer systems are reasonably similar and diverge markedly from a 1:1 ratio for C_{ew}/C_{pw} or C_b in favor of a relatively high C_{ew} value. Also shown are lines representing theoretical distribution of the drugs predicted on the basis of the intracellular–extracellular pH difference using a modified form of the Henderson-Hasselbalch equation (equation 5). The divergence of the experimental data from these lines appears to decrease in the order: bupivacaine > mepivacaine > lidocaine.

$$\frac{C_{in}}{C_{ex}} = \frac{1 + 10^{(pK_a - pH_{in})}}{1 + 10^{(pK_a - pH_{ex})}} \qquad (5)$$

where:

C_{in} = intracellular drug concentration, *i.e.*, C_{ew}

C_{ex} = extracellular free drug concentration, *i.e.*, C_{pw} or C_b

pH_{in} = intracellular erythrocyte pH(= 7.2[19])

pH_{ex} = extracellular pH (= 7.4)

(pK_a values are shown in table 6). It is assumed that only nonionized drug freely penetrates the erythrocytic membrane.

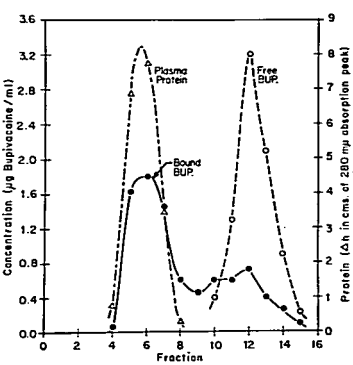

FIG. 2. Gel-filtration of bupivacaine added to human plasma and isotonic phosphate buffer, pH 7.4—one milliliter of plasma containing 5 μg of bupivacaine was eluted with isotonic phosphate buffer, pH 7.4. As a control, 1.0 ml of buffer containing 5 μg of bupivacaine was treated similarly. (Sephadex G-25 medium grade; 120 × 5 mm column; fraction size, 0.6 ml; room temperature.)

TABLE 4. *In Vitro* Plasma/Erythrocyte Drug Distribution Data*

	Hematocrit (Per Cent)	C_t	C_p	C_e	C_{ew}	Per Cent E	C_p/C_e	C_{pw}	C_{ew}/C_{pw}
Bupivacaine Subject 1	43.0	1.99	3.18	0.41	0.57	8.9	7.8	0.29	2.0
		5.00	7.70	1.43	1.99	12.3	5.4	1.55	1.3
		9.99	13.33	5.58	7.81	24.0	2.4	4.00	2.0
		19.73	23.49	14.73	20.62	32.1	1.6	10.60	2.0
Subject 2	46.0	2.00	3.56	0.39	0.55	9.1	9.4	0.35	1.6
		5.10	7.71	2.06	2.88	18.4	3.7	1.30	2.2
		9.71	12.59	6.31	8.83	29.9	2.0	3.98	2.2
		20.00	24.07	15.26	21.36	35.1	1.6	11.48	1.9
Mepivacaine Subject 1	43.0	2.02	2.76	1.05	1.47	22.2	2.6	0.71	2.0
		4.99	6.04	3.60	5.04	31.0	1.7	2.20	2.3
		10.01	11.42	8.15	11.41	35.0	1.4	5.93	1.9
		20.57	22.34	18.22	25.51	38.1	1.2	15.16	1.7
Subject 2	46.0	2.06	2.91	1.07	1.50	23.8	2.7	0.80	1.9
		4.96	6.61	3.20	4.23	28.0	2.2	2.58	1.6
		9.98	11.66	8.01	11.21	36.9	1.6	6.29	1.8
		21.04	23.26	18.43	25.80	40.3	1.3	16.89	1.5
Lidocaine Subject 1	43.0	2.00	2.57	1.25	1.75	26.8	2.1	0.96	1.8
		4.98	6.05	3.57	5.00	30.8	1.7	2.88	1.7
		10.00	11.39	8.16	11.42	35.1	1.4	6.49	1.9
		20.09	21.40	18.36	25.70	39.3	1.2	15.73	1.6
Subject 2	46.0	5.40	6.91	3.09	4.33	30.9	2.2	3.25	1.3
		10.80	12.65	8.62	12.07	36.7	1.5	7.53	1.6
		22.00	24.49	16.26	22.76	39.9	1.5	ND	ND

* C_t = total concentration of drug in blood. C_p = concentration of drug in plasma. C_e = concentration of drug in and/or on erythrocytes. C_{ew} = apparent concentration of drug in erythrocyte water. Per cent E = percentage of drug in and/or on erythrocytes. C_{pw} = concentration of drug in plasma water—all concentrations in μg/ml. ND = not determined.

† Value given to the nearest 0.5% and corrected for a nominal 3% trapped plasma.[18]

Distributions of the local anesthetics between erythrocytes and buffer of *p*H 7.4 as a function of total drug concentrations are shown in figure 4. As drug concentration decreases, the association of each drug with the erythrocytes, calculated as a percentage of total drug, tends to increase.

Partition Coefficients

The n-heptane/buffer partition coefficients (P and P') of the local anesthetics obtained using 20 and 100 μg/ml buffer drug concentrations and equations 2 and 3 are shown in table 6. Values decrease in the order: bupivacaine > lidocaine > mepivacaine > W-36017. Analysis of buffer solutions in the control tubes indicated negligible decomposition or loss of the drugs during the experiment.

Buccal Absorption Test

Results of these tests are summarized in table 6. The rank-order of absorption of the drugs was the same as that of their partition coefficients. No corrections were made for changes in volume and *p*H of the test solutions at the end of the tests. Such changes were of the order + 2 to + 5 ml and − 0.01 to − 0.05 *p*H units, respectively.

Discussion

Plasma Binding

Several methods for studying the binding of drugs to macromolecules are available, but unfortunately they all have disadvantages.[12, 26, 27] In view of this, when studying drug binding, it appears essential that at least two different

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experimental approaches be used. Centrifugal ultrafiltration was chosen as the main technique in the present study. A similar procedure has been used by others,[28–31] and it has the advantages of speed and simplicity. Equilibrium dialysis and gel-filtration methods were employed to provide semiquantitative confirmation of the extensive plasma-binding of bupivacaine observed in the ultrafiltration studies. The former method was also used to confirm the absence of significant binding of the drug to albumin and the latter method was used to give some indication of the strength of plasma binding.

In general, the concentration range considered in the ultrafiltration experiments included plasma drug levels anticipated after clinical administration of the local anesthetics. Some extrapolation of data to levels below 0.4 μg/ml is necessary, however, particularly in the case of bupivacaine. Our binding figures for lidocaine are much lower than those previously reported by others. Thus, Eriksson[6] found 66 ± 2% binding at the 10 μg base/ml level, and the figures of Shnider and Way[7] are consistently higher than ours over the range 2–10 μg/ml. At 2 μg/ml the latter apparently found 100% binding of the drug, although the statement of results from their equilibrium dialysis experiments is not compatible with their ultrafiltration data. Reynolds[32] has gathered ultrafiltration data which are in general agreement with ours.

Given the many subtle problems associated with the determination of plasma binding,[27] it would not be too surprising if the absolute values of the plasma-binding data quoted herein are in error to some degree. In criticism of the ultrafiltration technique, it is conceivable, for example, that the buffer controls did not adequately reflect the ability of the free drug *in plasma* to cross the dialysis membrane. Compounds not necessarily macromolecules present in plasma may have modified the permeability of the membrane or influenced the transport of free drug by complexation. Also, the ultrafiltration process may have altered the affinity of the drugs for binding to a plasma macromolecule. In a semiquantitative manner, however, the results with equilibrium dialysis and gel-filtration support the ultrafiltration data. Results with the latter technique suggest that the binding affinity of bupivacaine is quite strong, since the plasma–

TABLE 5. *In Vitro* Buffer/Erythrocyte Drug Distribution Data*

	C_t	C_b	Per Cent E	C_e	C_{ew}	C_{ew}/C_b
Bupivacaine						
Subject 1	1.05	0.74	57.7	1.52	2.13	2.9
	5.24	3.88	55.6	7.28	10.19	2.6
	10.14	7.85	53.6	13.59	19.03	2.4
Subject 2	1.10	0.78	57.6	1.58	2.21	2.8
	5.00	3.60	55.4	6.93	9.70	2.7
Mepivacaine						
Subject 1	1.02	0.83	50.9	1.29	1.81	2.2
	4.98	4.17	49.7	6.19	8.67	2.1
	9.97	8.66	47.9	11.94	16.72	1.9
Subject 2	1.00	0.83	50.3	1.26	1.76	2.1
	5.61	4.74	49.3	6.91	9.67	2.3
Lidocaine						
Subject 1	1.00	0.84	49.8	1.25	1.75	2.1
	5.11	4.35	48.9	6.25	8.75	2.0
	9.63	8.50	47.1	11.34	15.88	1.9
Subject 2	0.96	0.83	48.3	1.16	1.62	1.9
	5.00	4.38	47.4	5.93	8.30	1.9

* C_b = concentration of drug in buffer. All other symbols are explained in the footnote to table 4. All hematocrits adjusted to 40.0%.

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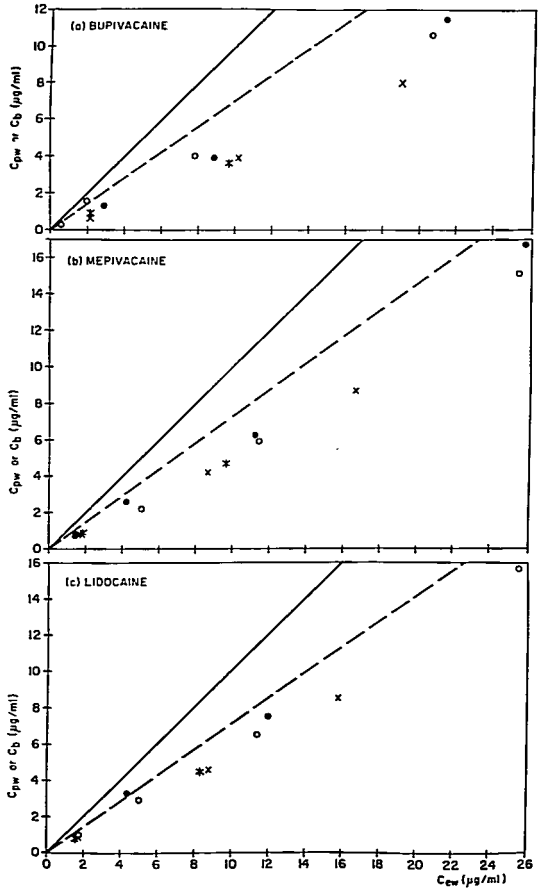

FIG. 3. Relationship (*in vitro*) between the concentration of free local anesthetic in plasma water (C_{pw}) or in buffer supernatant (C_b) and apparent concentration of drug in erythrocyte water (C_{ew}). (Plasma/erythrocyte data: circle, subject 1; solid dot, subject 2. Buffer/erythrocyte data: × = subject 1; * = subject 2. Solid lines represent 1:1 relationships. Broken lines represent theoretical relationships predicted on the basis of the intracellular-extracellular *p*H differentials.) *a*, bupivacaine; *b*, mepivacaine; *c*, lidocaine.

bupivacaine complex largely withstood the exhaustive dialysis effect anticipated under the conditions employed. In addition, indirect confirmation of our ultrafiltration results was obtained from the *in vitro* study of the distribution of the drugs between plasma and erythrocytes (see below).

Attempts to identify the plasma component responsible for binding the local anesthetics were unsuccessful. Conventional electrophoretic techniques proved unsuitable for this purpose. One of the major difficulties relates to dissociation of any drug-protein complex as the positively-charged drug molecules and the negatively-charged protein molecules migrate to opposite electrodes. Results with Cohn

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TABLE 6. Some Physicochemical and Biological Characteristics of Anilide-type Local Anesthetic Agents

	Molecular Weight of Base	pK_a (at 25C)	Partition Coefficient*						Per Cent Absorbed in Buccal Absorption Test				Plasma Binding‡ k (mM^{-1})
			a n-Heptane/Buffer				b Oleyl Alcohol/Buffer	c Oleyl Alcohol/Water	Subject 1	Subject 2	Subject 3	Mean	
			C_i = 20 μg/ml		C_i = 100 μg/ml								
			P	P′	P	P′							
Bupivacaine (racemic)	288	8.1[20]	27.5	156.1	21.7	137.5	314[20]	—	43.9 (48.5†)	36.3 (38.8†)	38.4 (37.5†)	38.1 (41.6†)	654
Mepivacaine (racemic)	246	7.65[21]	0.8	2.1	0.6	1.7	16.7[20]	6.7[25]	33.2	22.2	20.6	25.5	135
Lidocaine	234	7.86[22]	2.9	10.7	2.4	9.7	25[24]	12.5[25]	36.0 (38.5†)	25.6 (25.5†)	25.6 (25.5†)	29.1 (29.8†)	101
W-36017	206	7.36[22,23]	0.4	0.7	0.3	0.6	—	—	20.0	5.6	13.5	13.0	31

* *a*. Buffer *p*H = 7.43 (C_i = 20 μg/ml); 7.38 (C_i = 100 μg/ml). Temperature = 25 ± 2 C (*a.b.* *p*H values refer to buffer prior to addition of drugs).

C_i = initial concentration of drug (calculated as base) in buffer phase.
C_f = final concentration of drug (calculated as base) in buffer phase.

$$P = \frac{(C_i - C_f)}{C_f}; \quad P' = P(1 + 10^{pK_a - pH})$$

b. Buffer *p*H = 7.4 (bupivacaine and mepivacaine); 7.2 (lidocaine).
† Indicates result using a mixture of bupivacaine and lidocaine.
‡ k = affinity constant (see table 2).

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plasma protein fractions were inconclusive insofar as the isolated proteins may have been altered by the fractionation procedure, or a cofactor necessary for binding of the drugs may have been removed during preparation of the fractions. Also, the possibility that lipoproteins are involved in the binding cannot be excluded and is currently under further investigation. Significant plasma binding of the drugs apparently does not involve platelets.

Our findings, using both ultrafiltration and equilibrium dialysis, that insignificant amounts of lidocaine and bupivacaine are bound to albumin, contradict those of Sawinski and Rapp,[5] who reported extensive binding of lidocaine and mepivacaine to this protein at *p*H 7.0. It is difficult to reconcile these widely divergent conclusions, especially since Sawinski and Rapp used only 0.2% albumin solutions.

When studying plasma binding of basic compounds, it is particularly important to rule out any interaction with the anticoagulant used in blood, especially if this is heparin. The latter is a strongly acidic polysaccharide of large molecular weight and can, therefore, potentially form poorly-diffusible complexes with basic drugs. Indeed, tissue distribution of such drugs *in vivo* may be influenced by complexation with endogenous acidic mucopolysaccharides. This seems to be a reasonable possibility with regard to quaternary ammonium compounds, such as curare,[33] although our findings with serum and heparinized buffer suggest no significant interaction between heparinoids and local anesthetics.

Distribution in Blood

The correlation between values of C_{ew}/C_{pw} and C_{ew}/C_b (tables 5 and 6; see also figure 3) affords some support for the validity of the ultrafiltration data, from which values of C_{pw} were derived.

Discrepancies between C_{ew}/C_{pw} or C_b values and theoretical distribution of the drugs calculated using equation 5 indicate solution and/or binding of the drugs in or to erythrocytic components.* Some form of adsorption or binding is suggested by the trend towards increased distribution in and/or on erythrocytes with decrease in drug concentration, apparent in figure 4. This finding is not surprising in view of present knowledge of membrane pharmacology.[34, 35] For example, as part of extensive investigations involving the use of the erythrocytic membrane as a quantitative model for studying the molecular mechanisms of anesthesia, Kwant and Seeman[36] recently demonstrated the extensive adsorption of chlorpromazine (a potent local anesthetic) by erythrocytic ghost membranes.

Lipid Solubility

"Lipid solubility" is the term commonly used when interpreting the ability of compounds to penetrate (passively) into biological membranes and often, in addition, their ability to pass through such membranes. It is traditionally estimated by measuring the partition of compounds between water or buffer and a relatively nonpolar solvent. Clearly, the applicability to the biological system of partition coefficients obtained in this manner depends on the nature of the organic solvent used, *i.e.*, how closely its characteristics approximate those of the lipoprotein structures of biological membranes. Therefore, when comparing the lipid solubility of a series of compounds it is essential to measure partition coefficients in several different solvents in order to find trends which can also be expected to apply for biological membrane structures. Furthermore, although partition coefficients may reflect relative penetration of compounds into biological membranes adequately, the full relationship between partition coefficients and penetration of sets of compounds through such membranes is often parabolic in form.[37] This may be related to binding of compounds to components of biological membranes. Several systems have now been described which show linear relationships between partition coefficient and binding functions.[37]

In view of the above considerations, we have determined partition coefficients of the local anesthetics in the n-heptane/buffer system and compared them with data reported in the literature for the oleyl alcohol/buffer or water system (see table 6). Additionally, we have utilized the B.A.T., the chief advantage of which is the use of a biological membrane instead of an organic solvent. For a series of compounds, at constant buffer *p*H, the amount

* We assume that the process of separating cells from buffer or plasma does not affect equilibria involved in drug distribution between the blood components.

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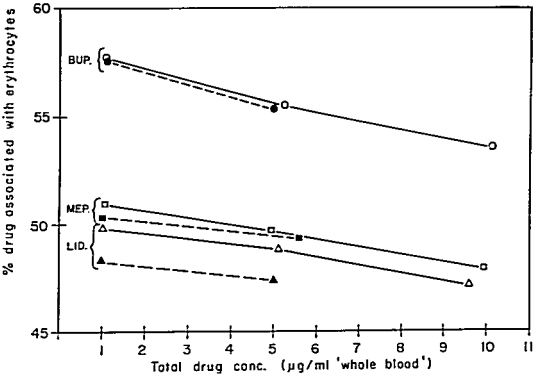

FIG. 4. Distribution of local anesthetics between erythrocytes and isotonic Sørensen's phosphate buffer, *p*H 7.4, as a function of drug concentration. [circles, bupivacaine (BUP); squares, mepivacaine (MEP); triangles, lidocaine (LID). Open symbols = cells from subject 1; closed symbols = cells from subject 2.]

of drug absorbed is assumed to be primarily a function of the pK_a of the drug and the rate of solution and partition coefficient of the nonionized form of the drug in buccal membranes. Drug binding may play a role subsidiary to drug partitioning in this system, in view of the lack of competition for uptake between lidocaine and bupivacaine when used together (table 6). Partition coefficients calculated from both of the distribution systems and the B.A.T. results make the same rank-order discrimination between the local anesthetics.

The n-heptane/buffer partition coefficients obtained at two different initial drug concentrations were similar, although the consistently slightly lower values for the higher concentration may reflect greater buffer *p*H displacement resulting from addition of the local anesthetic salts. Such *p*H changes may be small but significant.[22] Alternately, the differences could be accounted for by a degree of aggregation and/or interfacial concentration of the drugs. Several authors[38, 39] recently have demonstrated the ability of local anesthetics to exist in dimeric or polymeric forms and aggregates in solution, and such behavior may be significant even in terms of drug distribution and anesthetic activity.

Relationships Between Binding, Physicochemical Properties, and Anesthetic Activity

The data in table 6 indicate that the relative plasma-binding properties of the series of compounds studied bear no consistent relationship to either pK_a values or partition coefficients. Similarly, poor correlation between partition and protein binding in a series of ataractic drugs recently has been found.[12] Good rank-order correlation between binding and molecular weight is apparent, but this is probably not significant. That plasma binding is independent of steric configuration of the optical isomers of bupivacaine and mepivacaine (see table 3) suggests a relatively large degree of freedom for the interaction. In general, the rank-order of binding argues against the exclusive involvement of simple hydrophobic bonding, suggesting that specific structural features related to charges on the molecule are important. The same conclusion possibly also applies with regard to the association of drugs with the erythrocytic membrane. Although the data are less extensive, the rank-order of this association seems to parallel the plasma-binding affinity of the drugs (see figs. 3 and 4; table 6).

Several authors[34, 40, 41] have reviewed work which concludes that although the lipid solubility of a local anesthetic (as reflected by partition coefficients) affects nerve-blocking action, other factors undoubtedly are involved. These factors include the possibility of specific binding interactions between the anesthetic molecules and the membrane or other cellular components. It is of interest, therefore, to compare the rank-order of plasma binding (and erythrocyte binding) of the compounds studied with their anesthetic activity. In doing so,

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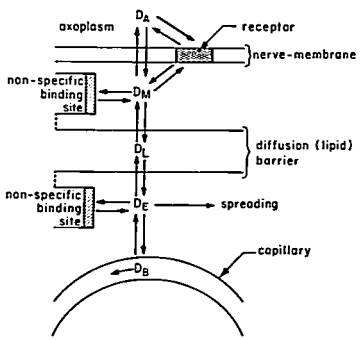

FIG. 5. Possible equilibria determining local anesthetic distribution near the site of action. A more complex scheme in which charged and uncharged forms of the drug are differentiated may be envisaged. (D_A = drug in axoplasm; D_M = drug in the vicinity of the membrane; D_L = drug in diffusion barrier; D_E = drug in extraneural tissue and fluid; D_B = drug in capillary blood.)

the question arises how best to express local anesthetic activity. Since "potency" is a rather complex concept, we have considered duration of anesthesia as being more likely to reflect "binding" affinity of the drugs in the biophase.

Factors potentially influencing duration of local anesthesia (fig. 5) include:

1) Intrinsic binding of drug to receptors in the nerve membrane.

2) Nonspecific binding of drug to membranes or proteins near the site of action.

3) Penetrability of drug to the biophase, mainly reflected by ability to pass into and through (lipoprotein) diffusion barriers.

4) Effect of drug on interstitial blood circulation near the site of action, by either a) intrinsic vasomotor activity, or b) indirectly, by an irritant effect causing increase in capillary permeability.

Attempts to relate the extent of plasma bindind of the compounds to possible binding at or near the nerve membrane, as reflected by activity data, therefore, are complicated by factors 3 and 4.

The rank-order of plasma binding is consistent with the general clinical impression that in humans bupivacaine is much longer-acting than mepivacaine,[42, 43] which, in turn, is marginally longer-acting than lidocaine.[44, 45] Furthermore, the duration of anesthesia produced by W-36017 in animal experiments is significantly shorter than that produced by lidocaine.[23] However, factor 4 may play a significant role in determining duration of anesthesia in these

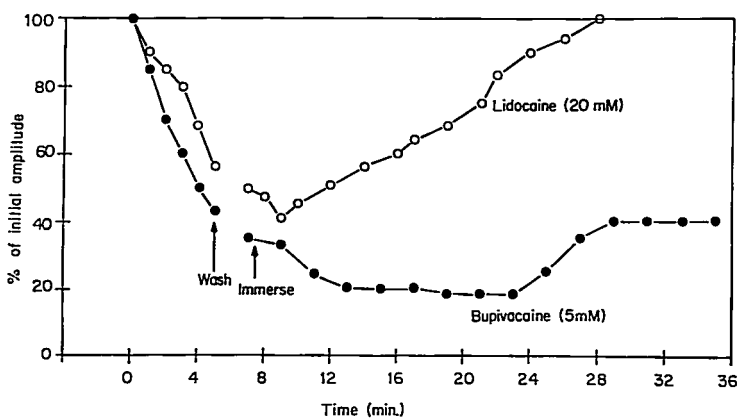

FIG. 6. Nerve block with lidocaine (20 mM) and bupivacaine (5 mM); pH7.[49] Isolated frog sciatic nerve preparation.

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systems, since the blood supply is intact. This also applies to simple animal tests such as the guinea pig intradermal wheal test.

Only factors 1 to 3 need be considered in studying the isolated frog sciatic nerve preparation.[46, 47] With this system, Truant and Wiedling[48] showed that when using equieffective concentrations of lidocaine and mepivacaine to produce nerve blocks, the recovery of the nerve is slower and not always complete with the latter compound. For both drugs, recovery times were prolonged compared with onset times, which may be interpreted as reflecting a more pronounced binding capacity and/or affinity of mepivacaine for neural components. This would be consistent with our plasma-binding data and inconsistent with lipid-solubility data. Results with bupivacaine are also in accord with the binding hypothesis. Prolonged recovery is observed using this compound (*c.f.*, results with lidocaine and bupivacaine shown in fig. 6). In this respect, bupivacaine is similar to other long-acting agents such as dibucaine (Nupercaine) and tetracaine (Pontocaine).[24, 47] For the latter, prolonged block in the isolated nerve system has been correlated with binding to various tissue homogenates, including that of sciatic nerve.[24]

Truant and Wiedling[48] also demonstrated that the onset of the block produced by mepivacaine was slower than that produced by lidocaine. This may be interpreted as being due to the lower lipid solubility of mepivacaine, resulting in slower penetration to the membrane receptors, or to the more extensive fixation of mepivacaine to nonspecific binding sites, thereby retarding its access to the receptors.[47] The greater uptake of lidocaine compared with that of mepivacaine in the B.A.T. perhaps could be taken as evidence that lipid solubility is the major factor determining onset of block, duration being primarily a function of binding.

Consideration of data obtained from experiments on isolated nerve appears to have separated factor 3 from factors 1 and 2. The question whether the durations of action of the compounds are related to binding at membrane receptors or to binding at nonspecific binding sites remains unanswered. Luduena[50] argued in favor of the predominant importance of the latter, although many of the theories of the mode of action of local anesthetics implicate specific interaction with lipid and/or protein components of the nerve membrane.[20, 34, 40, 51, 57] The binding of local anesthetics to phospholipids is particularly well documented.[52–56] It is tempting, therefore, to draw an analogy between binding of local anesthetics to plasma and erythrocytic components and binding to lipoproteins at or near the nerve membrane. Further work is necessary, however, to establish such an analogy and its possible use in the design of future local anesthetic agents.

The implications *in vivo* of the relative plasma-binding characteristics of the clinically-used agents, especially with respect to their transplacental passage, are considered in detail in Part II of this communication.

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Drugs

HYPERCARBIA FOR CAROTID SURGERY Carotid endarterectomies were performed without shunts in 137 patients. General anesthesia with methoxyflurane and *d*-tubocurarine was used, with CO_2 added. In 15 to 20 minutes the venous CO_2 tension reached 70 to 100 mm Hg without cardiac irritability or arrhythmias. In a previous series with cyclopropane, arrhythmias were a problem at this CO_2 tension. Advantages of the technique include lowered cerebral metabolism and oxygen requirement under general anesthesia; increased cerebral blood flow from the hypercarbia and hypertension; and avoidance of the encumbrance of the shunt apparatus. Good results were obtained in 93 per cent of patients. *(Young, J. R., and others: Carotid Endarterectomy without a Shunt, Arch. Surg. 99: 293 (Sept.) 1969.)*

HALOTHANE AND THE LIVER Wistar rats were anesthetized one hour daily for 30 days. No significant histologic changes were found in the liver, and electrophoretic and enzymatic test results were normal. *(Hartung, H., Oehmig, H., and Streicher, H. J.: Histological and Biochemical Results of Experimental Studies on Prolonged Halothane Action, Der Anaesthesist 18: 255 (Aug.) 1969.)*

DIAZEPAM Diazepam (Valium) was used intravenously for induction of anesthesia in more than 900 patients. It produced more profound amnesia than thiopental and reduced the amount of mucle relaxant required during the first hour of a laparotomy by 10 per cent. Postoperative nausea and vomiting occurred as frequently as after thiopental. *(Stovner, I., and Endresen, R.: Diazepam as an Induction Agent, Der Anaesthesist 18: 242 (Aug.) 1969.)*

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