

## Local Anesthetics and the Rheologic Behavior of Erythrocyte Suspensions

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The viscosity of a suspension of 45 per cent human erythrocytes in Ringer-albumin solution at 37 C was determined. Local anesthetics (hydrochloric salts of lidocaine, etidocaine, prilocaine, mepivacaine, bupivacaine, procaine, and tetracaine) were added to the erythrocyte suspension. In the presence of local anesthetics, the viscosity of the erythrocyte suspension at high shear rates was essentially constant. At low shear rates less than  $5 \text{ sec}^{-1}$ , the viscosity increased progressively as the anesthetic concentration was increased. Erythrocytes in the suspension were transformed progressively from discoid to cup-shaped as the concentration of anesthetic agent was increased. The transformation into the cup shape in the presence of local anesthetics may be attributed to the preferential expansion of surface area and increase in surface tension in the inner half-layer of the erythrocytic membrane. These morphologic changes of erythrocytes corresponded in general to the increase in viscosity of erythrocyte suspensions. The morphologic and viscosity changes were reversible by rewashing. The relative effectiveness of the anesthetic agents used, as estimated from the concentrations needed to increase viscosity at a shear rate of  $0.5 \text{ sec}^{-1}$  by 20 per cent, can be correlated with their relative intrinsic potencies, determined from isolated-nerve preparations. These data suggest that alterations in rheologic characteristics of erythrocyte suspension may be a useful index for the assessment of relative potencies of local anesthetics. The results further suggest that the inner half-layer of the membrane is the site of action for all the local anesthetics studied. (Key words: Anesthetics, local. Blood, erythrocytes: rheology, viscosity.)

LOCAL ANESTHETICS decrease the excitability of nerve membranes and produce conduction blockade. Local anesthetics also modify the physical properties of the membrane. Skou<sup>1,2</sup> has observed that local anesthetics expand the surface area and increase the surface pressure of a monomolecular layer of lipids extracted from nerves. Seeman<sup>3,4</sup> has reported that local anesthetics cause an expansion of erythrocytic membranes and protect erythrocytes from hypotonic hemolysis. In recent years, with the aid of electron spin resonance

technique, several investigators have demonstrated that local anesthetics fluidize lipid bilayers.<sup>5,6</sup> Hyperbaric pressure, which antagonizes the action of local anesthetics on nerve conduction, also prevents their fluidizing effects on membranes.<sup>7</sup>

The rheologic behavior of erythrocytes is dependent on, among other factors, the membrane properties of the cell. The present investigation was undertaken to examine systematically the rheologic effects of local anesthetics on suspensions of erythrocytes.

### Materials and Methods

The local anesthetics studied were hydrochloric salts of lidocaine, etidocaine, prilocaine, tetracaine, bupivacaine, mepivacaine, and procaine. The first three agents were obtained from Astra Pharmaceutical Products, Inc., Framingham, Mass., and the last four from Sterling-Winthrop Research Institute, Rensselaer, N. Y. Solutions containing various concentrations of local anesthetic were prepared by dissolving the hydrochloric salts of anesthetics in Ringer's solution containing 0.5 per cent (w/v) human serum albumin. The pH of the anesthetic solutions was adjusted to 7.4 with Tris buffer solution. At high concentrations, the anesthetic precipitated at pH near 7.4. Under these circumstances, the pH of the solution was decreased in order to maintain a clear solution. In all cases presented here, however, the pH value was not less than 7.0. The concentrations of anesthetics studied were: procaine 0.01 to 50 mmol/l; lidocaine, etidocaine, prilocaine, and mepivacaine, 0.01 to 20 mmol/l; tetracaine and bupivacaine, 0.01 to 10 mmol/l.

Fresh heparinized human erythrocytes were washed three times with Tris-Ringer-albumin solution (pH 7.4); albumin 0.5 per cent was added to the solution to prevent cellular crenation. The erythrocytes were resuspended at a hematocrit of 45 per cent in Tris-Ringer-albumin solution and in anesthetic solutions at various concentrations. The suspension was discarded whenever hemolysis was observed. The viscosities of the erythrocyte suspensions and the suspending media were measured in a co-axial cylinder

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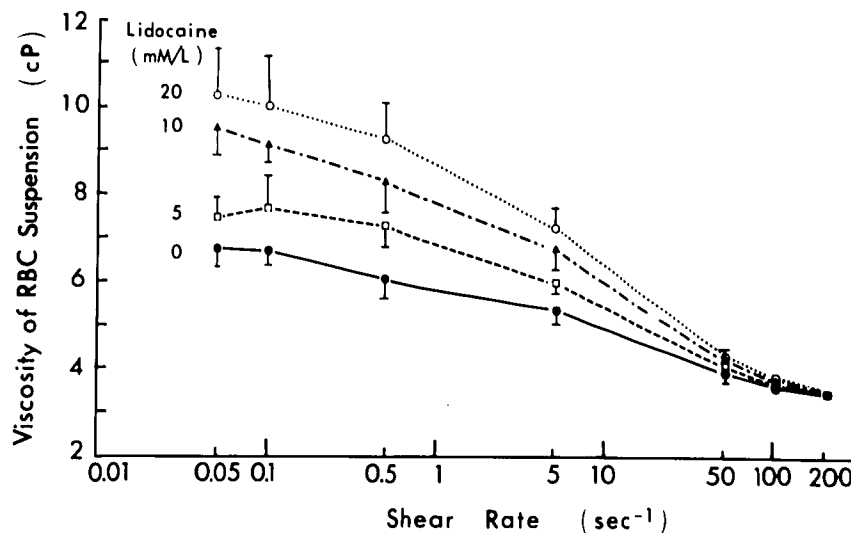


FIG. 1. Semilogarithmic plot of viscosity-shear rate curves of erythrocyte suspensions in various concentrations of lidocaine. The data are means  $\pm$  SEM of nine studies.

viscometer<sup>8</sup> modified from the original instrument devised by Gilson, Dauwalter and Merrill.<sup>9</sup> The mechanical components of the viscometer included two concentric cylinders separated by an annual gap of 960  $\mu$ m, where the sample was contained. The inner cylinder was rotated at a constant speed, whereas the outer cylinder, which rode on an air bearing, was held stationary by the torque generated from an electronic feedback system. The shear rates, calculated from the rotation speed and cylinder geometry, ranged from 200 to 0.01  $\text{sec}^{-1}$ . The shear stress was calculated from the torque generated with the use of a geometry-conversion factor, which was determined by employing viscosity-standard oils.<sup>§</sup> The viscosity of the sample was calculated as the ratio of shear stress to shear rate. The samples were always thoroughly mixed immediately before measurement. A guard ring was present at the air-sample interface. Measurements of viscosity were performed at 37 C. The erythrocyte count of the suspension was determined with a Coulter counter (Model B).<sup>¶</sup> Mean cell volume (MCV) was calculated from the hematocrit and the cell count. The osmolalities of suspending media were measured with an osmometer.<sup>\*\*</sup>

Following the measurement of viscosity, the erythrocytes in lidocaine suspensions were rewashed three times again with Tris-Ringer-albumin solution and resuspended in Tris-Ringer-albumin solution. The hematocrits of rewashed erythrocytes were adjusted to 45 per cent. Measurement of viscosity was repeated as described above.

For scanning electron microscopy, fresh erythrocytes and those treated with various concentrations of lidocaine were fixed in Tris-Ringer-albumin solution containing glutaraldehyde, 2 per cent, for one hour. The fixed cells were washed three times with distilled water and resuspended in distilled water at a hematocrit of approximately 0.5 per cent. Portions of the fixed, washed erythrocyte suspensions were air-dried on glass coverslips mounted on stubs and coated with gold-palladium in a Hummer 1 sputterer. The cells were examined and photographed with a scanning electron microscope (Jeolco Model JSM-25) at 15 kV and a 45-degree tilt.

### Results.

The viscosities of erythrocyte suspensions in various concentrations of lidocaine decreased as shear rates increased (fig. 1). For control erythrocytes suspension in Tris-Ringer-albumin solution, the viscosities at various shear rates agreed well with those previously reported.<sup>8</sup> In the presence of lidocaine, the viscosities of erythrocyte suspensions at high shear rates were essentially the same as the control viscosity (fig. 2). At low shear rates, viscosity increased progressively as the anesthetic concentration was increased. This effect was especially profound at shear rates of less than 5  $\text{sec}^{-1}$ . The osmolalities and the viscosities of the suspending media did not vary significantly at any concentration of anesthetic studied. The MCVs of erythrocytes in various suspensions were also essentially constant. Under scanning electron microscopic examination, erythrocytes in the suspension were found to transform progressively from discoid to cup-shaped as the concentration of anesthetic was increased (fig. 3). At lidocaine, 20 mmol/l, the erythrocytes were

<sup>§</sup> Cannon Instrument Co., Boalsburg, Pa.

<sup>¶</sup> Coulter Electronics, Hialeah, Fla.

<sup>\*\*</sup> Fiske Associates, Inc., Bethel, Colo.

predominantly stomatocytes. These morphologic alterations of erythrocytes can be related to the changes in viscosity of erythrocyte suspensions. Rewashing experiments showed that both the morphologic and viscosity changes were reversible. Similar results were found for all other local anesthetics examined.

The effects of local anesthetic agents on the viscosity of erythrocyte suspension at a shear rate of  $0.5 \text{ sec}^{-1}$  were compared by using the value for an erythrocyte suspension in Tris-Ringer-albumin solution as the control. Per cent change in viscosity was plotted against anesthetic concentration, and the resulting dose-response curves for various anesthetics were generally parallel to one another (fig. 4). The relative effectiveness of these anesthetics were assessed by comparing the concentrations needed to cause a 20 per cent increase in viscosity (shear rate =  $0.5 \text{ sec}^{-1}$ ) of erythrocyte suspensions (table 1). Procaine was the least potent in increasing the viscosity of the erythrocyte suspension. Lidocaine, prilocaine and mepivacaine were of intermediate potency, while etidocaine, tetracaine and bupivacaine were the most potent. The relative effectiveness of anesthetics in inducing changes in the viscosity of the erythrocyte suspension correlated well with their relative potencies in decreasing the amplitude of A-spike action potentials of isolated frog sciatic nerve (fig. 5).<sup>10</sup>

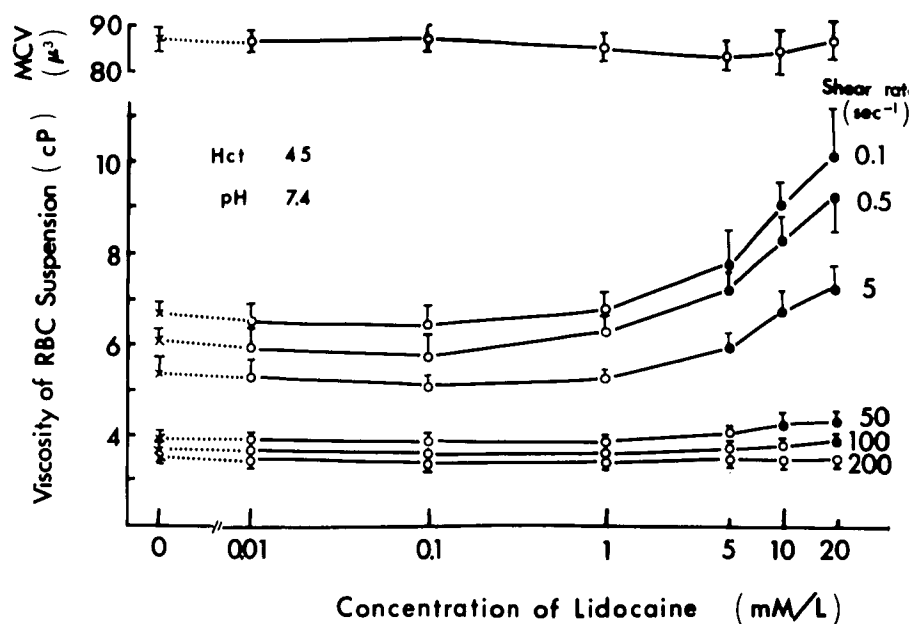
### Discussion

The rheologic behavior of blood or erythrocyte suspensions at a given temperature is a function of

concentration of cells, the viscosity of plasma (or suspending medium), cellular aggregation, and changes in cellular shape.<sup>11</sup> In the present investigation, the erythrocyte concentration was adjusted to 45 per cent in all experiments. The viscosities of the suspending media were essentially constant. With the use of suspensions of erythrocytes in buffered Ringer's solution, cellular aggregation was eliminated. The viscosities of erythrocyte suspensions measured in a co-axial cylinder viscometer at various shear rates thus reflect the morphologic changes of erythrocytes in response to the hydrodynamic stress generated by the rotation of the inner cylinder.

Local anesthetics cause an increase in the viscosity of erythrocyte suspensions at low shear rates that may result from alteration of membrane properties or a change in the geometry of erythrocytes.<sup>12</sup> In the anesthetic suspensions, the erythrocytes were transformed progressively into stomatocytes as the anesthetic concentration increased, and these changes in erythrocytic shape could be correlated with the increases in the low-shear-rate viscosities of the suspensions. The viscosities at high shear rates were found to be essentially the same for all suspensions. Thus, erythrocytes in the presence of local anesthetics can be deformed to a hydrodynamic shape comparable to that of normal cells when the deforming stress is sufficiently high. Similar rheologic behavior has been found in ATP-depleted human erythrocyte suspensions.<sup>13</sup> The present investigation emphasizes the importance of cellular morphologic characteristics in interpreting the results of viscometric flow behavior.

FIG. 2. Semilogarithmic plot of viscosities of erythrocyte suspensions against concentrations of lidocaine in the suspending media at various shear rates (*lower panel*). The upper panel denotes the mean cell volume (MCV) in the suspension. The data are means  $\pm$  SEM of nine studies. The closed circles indicate that the difference between the experimental value and the control value is statistically significant by the Student *t* test ( $P < 0.05$ ).



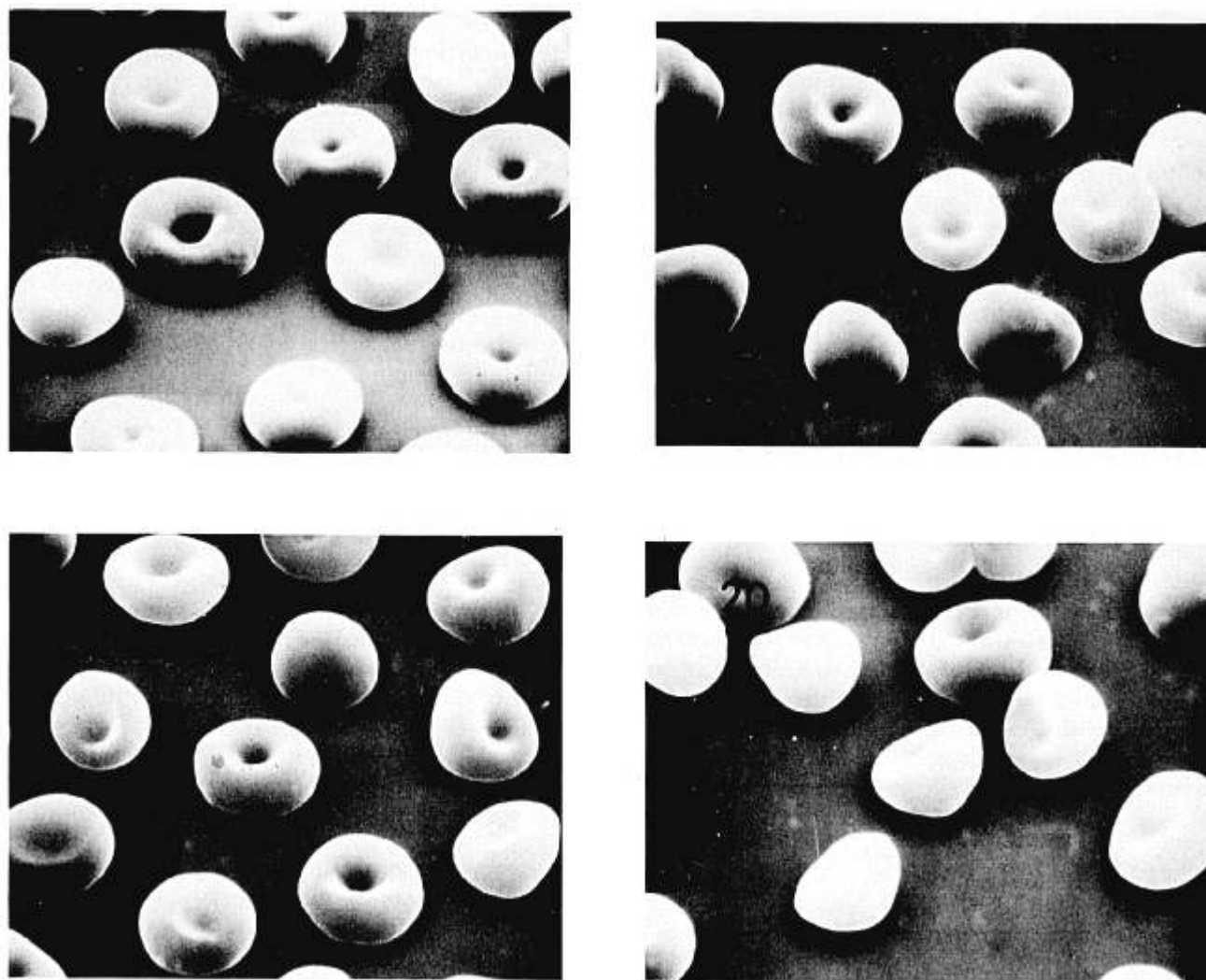


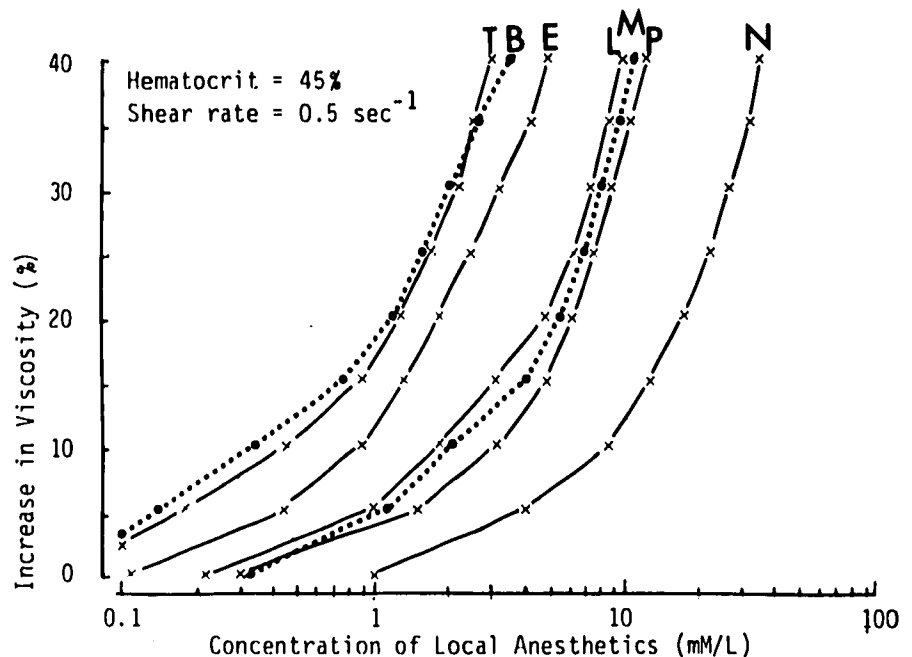
FIG. 3. The appearances of erythrocytes in suspensions of various concentrations of lidocaine under the scanning electron microscope ( $\times 4,500$ ). The number on each graph indicates the concentration of lidocaine in mmol/l. Notice that the shape of the erythrocytes was transformed progressively from biconcave, discoid into cup-shaped as the concentration of lidocaine was increased to 20 mmol/l.

Isolated nerve preparations *in vitro* have been employed by most investigators to determine the conduction-blocking activities of specific local anesthetics.<sup>14-16</sup> The relative potencies of different local anesthetic agents are obtained by comparing the minimal concentrations needed to block impulse conduction in a nerve fiber of a given diameter within a specific period.<sup>17</sup> The standardization of nerve preparations and the procedure for determining conduction blockade, however, are usually tedious and difficult.<sup>10</sup> The results of the present study suggest that the rheologic characteristics of erythrocyte suspensions in local anesthetics may be a useful index for assessing the relative potencies of these agents.

The mechanism of the morphologic alterations of erythrocytes induced by local anesthetics is not clearly understood. There is evidence that biological mem-

branes behave like bilayer couples.<sup>18,19</sup> The two halves of the bilayer membrane differ in their protein and lipid constituents, and can respond differently to membrane perturbation. If the membrane forms a closed surface, as in the intact erythrocyte, a change in the relative surface areas of the two halves leads to alterations in the curvature of the membrane. On the basis of a theoretical treatment of bending moments and membrane tensions of membrane bilayers, Evans has suggested that alterations in the surface tension of the inner and outer half-layers can create bending moments and induce profound curvature changes.<sup>20</sup> An expansion in the surface area in the outer half-layer of the membrane leads to an increase in surface tension and causes the erythrocyte to crenate. When the same perturbation occurs in the inner half-layer, the resulting erythrocyte is cup-shaped.

FIG. 4. Dose-response curves for various local anesthetics studied. The ordinate indicates the per cent increases in viscosities of erythrocyte suspensions for each anesthetic agent above the control values. B = bupivacaine; E = etidocaine; L = lidocaine; M = mepivacaine; N = procaine; P = prilocaine; T = tetracaine.



Deuticke has demonstrated that most of the negatively charged agents cause the erythrocyte to crenate, while positively charged agents cause the erythrocyte to assume an invaginated, or cup, shape.<sup>21</sup> All these drugs bind the membrane by intercalating their hydrophobic portions into the lipid bilayer with their ionic heads in the membrane surface. Local anesthetics in their charged cationic form would electrostatically interact with the negatively charged phospholipids, *e.g.*, phosphatidylserine and phosphatidylethanolamine, which are largely confined to the inner half-layer of the erythrocytic membrane.<sup>22-25</sup> This would preferentially increase the fluidity and expand the area of the inner half-layer of the membrane, thus producing cup-shaped erythrocytes. It is of special interest that local anesthetics may exert a similar effect on neural membranes. Evidence has been provided by several investigators that tertiary local anesthetics are most

potent in their cationic form acting from the inside of the nerve.<sup>16,26,27</sup> The uncharged form may be responsible mainly for the diffusion through the nerve sheath. The present study, however, cannot rule out the possibility of specific receptors associated with sodium channels in the nerve membrane for local anesthetics, as proposed by Strichartz<sup>28</sup> and Hille.<sup>29</sup>

TABLE 1. Concentrations of Local Anesthetics Needed to Produce a 20 Per Cent Increase in Viscosity of Erythrocyte Suspensions (Shear Rate = 0.5 sec<sup>-1</sup>)

	Concentration (mmol/l)	Relative Effectiveness in Viscosity Change	Relative Potency in Conduction Block*
Procaine	13.5	0.37	0.25
Prilocaine	6.03	0.83	0.75
Mepivacaine	5.75	0.87	0.50
Lidocaine	5.01	1	1
Etidocaine	1.91	2.62	4
Tetracaine	1.35	3.7	4
Bupivacaine	1.25	4.0	4

\* The data of relative potencies in conduction block are taken from Covino and Vassallo.<sup>10</sup>

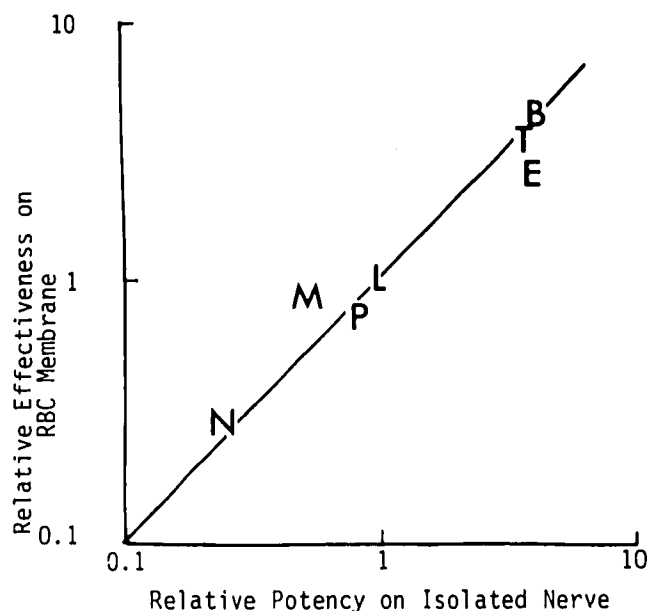


FIG. 5. Correlation of relative potencies of local anesthetics in causing rheologic effects on erythrocyte suspensions and blocking nerve conduction in isolated nerve. B = bupivacaine; E = etidocaine; L = lidocaine; M = mepivacaine; N = procaine; P = prilocaine; T = tetracaine.

In common clinical practice, the highest blood levels following an average dose of 400–500 mg of local anesthetic for lumbar epidural anesthesia was found to be about 4–5  $\mu\text{g/ml}$ , or 0.01–0.02 mmol/l.<sup>30,31</sup> Since the viscosities of erythrocyte suspensions and the morphologic characteristics of erythrocytes remain essentially unchanged at these concentrations of local anesthetics, the usual dosage used in clinical anesthesia does not affect erythrocytes in circulating blood. The tissues, including neural membranes, in the vicinity of the injection site, however, are bathed in anesthetic solution at concentrations (10–80 mmol/l) comparable to those causing rheologic effects on erythrocyte suspensions. The results of our study substantiate the point of view that local anesthetics alter physical properties of the biological membrane.<sup>32,33</sup> The morphologic changes of erythrocytes suggest that local anesthetics expand the inner half-layer of the erythrocytic membrane. Further studies are needed to determine the effects of local anesthetics on physical properties of the neural membrane, in order to generalize the mechanism of conduction blockade.

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