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PENETRANCE OF LOCAL ANESTHETICS *

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Most of the studies of local anesthetics have given little consideration to the diffusibility of the compounds from the point of application to the site of action. The investigations in Kato’s laboratory (1) have demonstrated that the sites of action of local anesthetics on myelinated fibers are the nodes of Ranvier. They also established that in single, isolated, frog sciatic nerve fibers, very weak solutions of cocaine (0.025 per cent to 0.005 per cent in Ringer’s solution) applied on a Ranvier node produced conduction block within one second. These strengths of cocaine are ineffective when applied on the rabbit cornea or the intact frog sciatic nerve. If progressively greater concentrations are tested, a certain strength of cocaine will be found which produces corneal anesthesia and another which blocks conduction in the sciatic nerve of the frog. These threshold concentrations require much longer than one second to induce anesthesia. The time of induction is shortened if more concentrated solutions are used. In the light of the results reported by Kato, the time of induction represents the time required for the local anesthetic to diffuse from the site of application to the site of action.

This point is further exemplified in the experiments carried out by Wilbrandt and de la Caudra (2) on the frog sciatic nerves. The time of induction and the duration of the conduction block were greatly shortened by longitudinal dissection and separation of the fiber bundles in the area where the local anesthetic was applied.

In the clinical usage of local anesthetics, especially when the agent is injected around a large nerve or intraspinally, the desired degree of anesthesia is obtained only after a period of time that is measured in minutes. As an inherent property, some compounds are known to induce anesthesia more rapidly than others. A short induction time with clinical doses is spoken of as equivalent to rapid penetration and the compound is said to be a good penetrant. This type of compound is used preferentially in dentistry, and in nerve block and infiltration anesthesia. Those compounds which produce anesthesia after a longer period of induction are used mainly in spinal anesthesia and anesthesia of the cornea or mucous membranes; in both cases the amount of inert

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tissue between the anesthetic depot and the nerve fibers (or nerve endings) is very small. They are used in spite of a long induction time because they invariably produce anesthesia of long duration.

In clinical as well as in experimental anesthesia, the speed of induction with a given anesthetic depends on: (1) local anesthetic activity, (2) dose (volume and concentration of the solution injected), (3) distance from the site of application to the site of action, (4) penetrability of the tissues between those two sites, and (5) the penetrance of the compound or, in other words, its inherent ability to penetrate those tissues. In clinical nerve block, the anesthetic solution must first reach the nerve that is to be anesthetized. The initial concentration is gradually reduced by mixing with the tissue fluids and, other things being equal, the greater the distance of the nerve from the site of infiltration, the greater the dilution. The original concentration is further reduced in that some of it is inevitably carried away in the lymph and the blood. This means that the anesthetic has to be injected at concentrations much higher than the true threshold anesthetic concentration for the particular nerve fibers involved. Reduction of the amount of blood passing through the chosen area by adding a vasoconstrictor drug to the solution causes a greater duration of anesthesia and increases the effective time in which penetration may be obtained.

Penetration of local anesthetic agents has been determined in the sciatic nerve of the cat in vivo. We could not use, as will be shown subsequently, the same concentration with all the drugs because of important differences in local anesthetic activity. It is obvious that if a hypothetical compound A is fifty times more active than compound B, and both are tested at the same concentration, the same degree of nerve paralysis will be produced faster with compound A. We have used, therefore, drugs in equiactive concentration in these experiments, that is, concentrations in inverse ratio to their local anesthetic activity. This requires a reliable method for the determination of activity. The intracutaneous wheal method of Büllbrin and Wajda (3) was found to be satisfactory. The rate of penetration of the anesthetic compound does not appear to be a major factor in the results obtained by this technique. The method of estimating activity and the results obtained will be published elsewhere (Luduena and Hopkins, 4). The present paper summarizes the results of a survey made with five local anesthetic compounds in which nerve penetrance and recovery time have been compared. The procedure is somewhat similar to that used by Bennett et al. (5) and Ehrenberg (6) on the isolated frog sciatic nerve preparation.

**Materials and Methods**

Each compound, identified by its structural formula and corresponding WIN number or name is shown in table 1. The local anesthetic potency as determined by the sciatic nerve block in guinea pigs, the
<table>
<thead>
<tr>
<th>Compound and Structural Formula</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean ± s.e.</th>
<th>Mean Recovery Time, min.</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean ± s.e.</th>
<th>Mean Recovery Time, min.</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean ± s.e.</th>
<th>Mean Recovery Time, min.</th>
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<td><strong>Procaine</strong></td>
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<td>NH$_2$</td>
<td>0.8</td>
<td>73±9.3</td>
<td>28</td>
<td>3.4</td>
<td>45±10.3</td>
<td>24</td>
<td>1.7</td>
<td>38±8.8</td>
<td>16</td>
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<td>C$_2$H$_5$HCl</td>
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<td>COOCH$_2$CH$_3$N$_2$C$_2$H$_5$</td>
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<td><strong>Ravocaine®</strong></td>
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<td>NH$_2$</td>
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<td>73±9.7</td>
<td>40</td>
<td>0.375</td>
<td>47±7.5</td>
<td>41</td>
<td>0.187</td>
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<tr>
<td><strong>Lidocaine</strong></td>
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<td>CH$_3$</td>
<td>1.74</td>
<td>68±8.3</td>
<td>33</td>
<td>0.87</td>
<td>58±10.9</td>
<td>26</td>
<td>0.435</td>
<td>23±5.2</td>
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**Penetration of Local Anesthetics**
<table>
<thead>
<tr>
<th>Compound and Structural Formula</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean±s.e.</th>
<th>Mean Recovery Time, min.</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean±s.e.</th>
<th>Mean Recovery Time, min.</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean±s.e.</th>
<th>Mean Recovery Time, min.</th>
<th>Conc., per cent</th>
<th>Per cent Blockade Mean±s.e.</th>
<th>Mean Recovery Time, min.</th>
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<tr>
<td>WIN 5760</td>
<td>0.106</td>
<td>85±5.7</td>
<td>240-360</td>
<td>0.053</td>
<td>75±4.2</td>
<td>180-240</td>
<td>0.026</td>
<td>44±4.2</td>
<td>60-150</td>
<td>0.0134</td>
<td>22±4.2</td>
<td>60-90</td>
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<tr>
<td>Dibucaine</td>
<td>0.474</td>
<td>44±6.8</td>
<td>+360</td>
<td>0.237</td>
<td>38±4.5</td>
<td>+360</td>
<td>0.168</td>
<td>27±3.6</td>
<td>180-240</td>
<td>0.084</td>
<td>15±2.4</td>
<td>120-180</td>
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</tbody>
</table>
Penetration of Local Anesthetics

Toxicity and irritancy of WIN 3459 (ravocaine®†) have been reported [Luduena and Hoppe (7)]. The high activity and especially the high activity/irritancy ratio of ravocaine® when compared to procaine and other known local anesthetics are its most characteristic features. WIN 3766 is a member of a new series of local anesthetics studied in this laboratory and is characterized by its extremely high activity, especially when used as a topical anesthetic.

The relative values reported in Table 2 for local anesthetic activity were obtained by Luduena and Hoppe (4) using the method of Bulbring and Wajda (3). The method for determining irritancy by intradermal injection in rabbits as well as the values obtained for procaine and ravocaine®, which are given in Table 2, have been published (7).

**Table 2**

Relative Local Anesthetic Activity and Irritancy

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity Ratio*</th>
<th>Irritancy Ratio</th>
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<tr>
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<td>In Terms of</td>
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<tr>
<td></td>
<td>the Base**</td>
<td>the Base**</td>
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<tr>
<td></td>
<td>Molar</td>
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</tr>
<tr>
<td>Procaine</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Ravocaine</td>
<td>8.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Lidoceine</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>WIN 3766</td>
<td>75</td>
<td>8.9</td>
</tr>
<tr>
<td>Dibucaine</td>
<td>15</td>
<td>22</td>
</tr>
</tbody>
</table>

* Determined by the guinea pig intradermal wheal method of Bulbring and Wajda (3) (Luduena and Hoppe, 4).

** These activity ratios are very close to those estimated for the hydrochloride salts.

All solutions used were in normal saline adjusted to pH 5.5 to 6.5 with sodium hydroxide and hydrochloric acid. The cats selected were of either sex and an attempt was made to use only cats weighing from 2.0 to 3.0 kg. in order to reduce the range of nerve diameter. Altogether 64 cats were used of which 4 were above 3.0 kg. in weight (3.5 to 4.1 kg.) and 8 were below 2.0 kg. (1.7 to 1.9 kg.). All animals were anesthetized with sodium pentobarbital, 32 mg. per kilogram of body weight, intraperitoneally and sustained under anesthesia with intravenous pentobarbital as needed.

The cats were placed in a prone position upon a supporting rack with the posterior limbs immobilized at the knees. The Achilles tendon was exposed, severed and attached to the lever arm of an isometric muscle lever. The sciatic nerve was exposed through the posterior surface of the thigh, severed at the proximal end near the pelvic girdle and drawn through a second smaller opening in the lateral distal aspect of the thigh. The nerve was then drawn into a fluid electrode [Ross (8)] with a volume of 2 cc. and fixed in position. Once in place, the nerve was bathed in Ringer-Locke solution.

† WIN 3459 is now being marketed under the trade name of Ravocaine by Cook-Waite Laboratories, Inc.
The nerve was excited with a simple square wave stimulator (A. H. Thomas Co.) at an E. M. F. of approximately 2 volts (more than twice the excitation threshold) every four seconds for a pulse duration of one-thousandth second. As soon as a standard contraction was obtained, the Ringer-Locke solution surrounding the sciatic nerve was withdrawn and replaced with approximately 2 cc. of the local anesthetic solution in normal saline. Stimulation was again commenced and the penetration of the anesthetic was measured by the blockade of the isometric muscle twitch. Notation of the percentage of blockade was made at ten and fifteen minutes following application of the drug, at the end of which time the local anesthetic was withdrawn from the electrode chamber. The nerve was washed twice within a period of one minute with Ringer-Locke solution and stimulation again initiated. At the end of a five minute interval of stimulation following the last

washing, the nerve was washed a third time and then allowed to continue to complete recovery. Recovery was judged complete when the muscle twitch had returned to the same magnitude of contraction as that preceding medication. If the blockade of impulse transmission was complete within less than fifteen minutes, the anesthetic was withdrawn at the time and the nerve washed (fig. 1).

It was found during the course of these experiments that if a second determination with the same concentration was made on a nerve following complete recovery of the muscle twitch amplitude, greater or faster block was observed in the second determination. This introduced a source of error and precluded the use of the same nerve for more than one test for quantitative determinations. Therefore, all the values reported in table 1 were the results of the first test on each nerve except in a few cases in which a second test was included which
followed medication at the lowest dose range and where sufficient time was allowed between treatments (approximately four hours) so that first treatment did not interfere with the second treatment.

Four dose levels, graded at 0.3 logarithmic intervals, were used and eight determinations were made for each point. The concentrations of procaine hydrochloride were: 0.25 M, 0.125 M, 0.0625 M and 0.03125 M. Procaine was tested in parallel with dibucaine hydrochloride (nupercaine, Ciba). Each concentration of procaine was tested on one sciatic nerve of the cat and on the opposite sciatic nerve an equiactive concentration of dibucaine was applied. The equiactive concentration of dibucaine was calculated by dividing the corresponding molar concentration by the molar procaine activity ratio of dibucaine as determined by the Bülbbring and Wajda method (4) using the ratios of activity summarized in table 2. For instance, in terms of mols, dibucaine is twenty times more active than procaine. In order to compare equiactive concentrations, the 0.25 M concentration of procaine hydrochloride was tested against the \( \frac{0.25}{20} \) M concentration of dibucaine. Ravocaine® and lidocaine (xylocaine, Astra) were tested on the same cats in the same manner in equiactive concentrations and WIN 3766 was tested alone at equiactive concentrations.

Results

In determining the penetrance of the compounds used in this investigation, no attempt was made to determine the threshold concentration of anesthesia or the concentration which gave complete blockade within ten minutes. The dose range used, however, does show a representative spread of the penetrance.

The degree of sciatic nerve blockade, as measured by the decrease of amplitude (or suspension of muscular contraction), expressed in percentages, is shown with the corresponding standard errors in table 1. The magnitude of inhibition produced by each compound as measured at ten minutes after application, increases with the concentration. This response bears a linear relationship to the logarithm of the concentration within the range of concentration used (fig. 2).

Procaine, lidocaine and ravocaine®, in equiactive concentrations, produced the same degree of blockade in the same period of time. We may conclude that they have approximately the same degree of nerve penetrance. Dibucaine has a much lower penetrance, as shown by the slope of the dose-response curve. On the other hand, WIN 3766 produced higher degrees of blockade at all concentrations. Its dose-response curve has the greater slope. The average effect obtained with 0.03125 M procaine and the equiactive concentrations of the other compounds tested were approximately of the same magnitude with the exception of WIN 3766 which was somewhat greater. This may be ex-
plained by the fact that at the lowest concentration tested, the effect of the local anesthetics during the short period of observation (ten minutes) is restricted to the most superficial fibers of the nerve. Any difference in the speed of penetration cannot be observed clearly until the compounds have traveled a greater distance toward the center of the nerve. This was accomplished by increasing the concentration. It might also have been accomplished by increasing the observation time from ten minutes to, say, one hour while keeping the concentrations constant.

![Graph showing dose-response curves of local anesthetics](image)

**Fig. 2.** Dose-response curves of procaine; ravocaine® (WIN 3459), lidocaine, WIN 3766 and dibucaine. Percentage blockade of amplitude has been plotted on the ordinate while molar procaine concentration and procaine molar equivalent concentration of the other drugs has been plotted on the abscissa. See text for explanation.

The time for complete recovery varied for each compound. The rate of recovery occurred in the following sequence: procaine > lidocaine > ravocaine® > WIN 3766 > dibucaine (fig. 3). The average recovery time in minutes after application of the highest concentrations was as follows: procaine twenty-eight minutes; lidocaine, thirty-three minutes and ravocaine®, forty minutes. It took a much longer time for the nerve to recover after WIN 3766 (four to six hours), while the effect of the highest concentration of dibucaine did not disappear completely within a period of six hours.
An attempt has been made in this investigation to determine the ability of some local anesthetics to diffuse into and out of nerve tissue when applied topically in various concentrations. The studies of Bennett et al., Ehrenberg and others have established that the time required for induction of nerve block on isolated nerves varies inversely with the concentration and directly with the square of the radius of the nerve. We have used animals of approximately the same size and,

in comparing ravenocaine® with lidocaine and procaine with dibucaine, the factor of varying nerve size has been minimized by testing the drugs on the same cats. The influence of local anesthetic activity and dose, two conditions enumerated in the introduction as factors on which penetration depends, have been eliminated or minimized by using equiactive doses of the compounds. If equal concentrations are used the most active compound has a greater chance of producing the greatest degree of paralysis in a given time. For instance, 0.474 per cent dibucaine hydrochloride produced a more pronounced effect than 6.8
per cent procaine hydrochloride or 1.7 per cent lidocaine (table 1). This is easily explained by assuming that the amount of drug required to paralyze the fibers in the nerve is much smaller with dibucaine and, therefore, this effective concentration would be obtained faster after topical application of the same percentage concentration.

The use of equineactive concentrations introduces the error inherent in the method of determining activity. Figure 2, however, shows that the dose-response curves of the compounds tested either cross or are close to crossing at points below the 20 per cent blockade value. This indicates that at the lowest concentration used the local anesthetic activities are about the same. More important for the estimation of penetrance is the slope of the dose-response curve. As mentioned previously, the higher the penetrance the greater the slope.

Our results indicate that penetrance is not related to activity. Procaine, lidocaine and ravocaine® show equal penetrance, but their activity differs from 1 (procaine) to 11 (ravocaine®), on a molar basis. Moreover, of the two most active compounds, dibucaine has the lesser and WIN 3766 has the higher penetrance.

Molecular weight is another factor which may affect penetrance. In view of the similarity in behavior of molecules in dilute solutions and in the gas phase, it has been inferred from Graham’s law that diffusion of solutes is inversely proportional to the square root of the molecular weight. This may be a factor that contributes to the penetrance values we have obtained, but if so it could not be of great importance considering the relatively small differences in the molecular weights.

Irritancy of the compounds may be negatively correlated with penetrance. Again we must consider not the absolute irritancy, but the irritancy of the concentrations used, or in other words, the activity/irritancy ratios. The least penetrant of the compounds tested, dibucaine, had the lowest activity/irritancy ratio while the most penetrant, WIN 3766, had the highest activity/irritancy ratio, that is, the least relative irritancy. This may be an indication that there is a negative correlation between relative irritancy and penetrance, but many more compounds will have to be tested in order to establish the existence or absence of any such correlation.

An unexpected finding was the lack of correlation between the speed of penetration and the recovery time. It might have been assumed that if one compound diffused into the nerve at a faster rate than a second compound, it would also diffuse out more rapidly than the latter. This, however, was not the case. WIN 3766, which penetrated the nerve at a faster rate than procaine, ravocaine® or lidocaine, remained in the nerve a much longer time than any of them, as judged by the recovery time. This may be an interesting property when rapid onset and relatively long duration of action are required of an anesthetic agent.
The recovery time was shortest following treatment with procaine. It was somewhat shorter with lidocaine than with raxocaine® and was the longest (several hours) with dibucaine, a fact which agrees with clinical observation. No explanation can be offered at this time for the various rates of recovery. However, if dibucaine is omitted and the other compounds considered as a group, all of these being much less irritating than dibucaine, it may be noted that within the group the weaker the compound the shorter the recovery period. Local destruction of the anesthetics may play a part in decreasing their concentration to subthreshold values. Dibucaine is not destroyed by pseudocholinesterase and this may be a contributory factor to the long period of recovery. Procaine, on the other hand, is hydrolyzed by procainesterase (most likely pseudocholinesterase). We have no information on the effect of pseudocholinesterase on raxocaine® and WIN 3766.

As mentioned before, a second application of a drug at the same concentration given immediately following apparent complete recovery produced a much greater effect. For this reason, only the effect of first applications was used in the determination of the penetrance with the exceptions as noted previously. A possible explanation of this phenomenon may be as follows: during the recovery time the amount of anesthetic is constantly decreasing. Sometimes it was observed that when the effect was intense but the blockade was not complete at the end of the period of exposure, complete nerve block did occur two or three minutes after the bathing anesthetic solution had been replaced by Ringer-Locke solution. This can be simply explained by assuming that diffusion of the anesthetic toward the center of the nerve continued after washing. During the recovery time the local anesthetic diffuses out and the original amplitude is reached when the concentration throughout the nerve is below the threshold value. When the nerve is again exposed to a solution of local anesthetic shortly after the end of the recovery period, a smaller amount of anesthetic has to pass into the nerve to raise again the intraneural concentration above the threshold value. In our experiments, when the second determination was carried out two to four hours after recovery, the values were approximately equal to those obtained with the first test, suggesting—that no residual anesthetic remained inside the nerve two to three hours after the first test or that the amount was negligible.

A similar phenomenon has been reported by Holmes and co-workers (9), that is, that the effect of d-tubocurarine on the isolated rat phrenic nerve-diaphragm preparation was progressively greater in succeeding tests. A residuum of d-tubocurarine in subthreshold amounts may have been a factor, but not the only one in this sensitization of the preparation to repeated tests with d-tubocurarine.
Summary

Five local anesthetics have been tested in vivo for the rate of penetration on the sciatic nerve of cats as measured by the amplitude of the gastrocnemius twitch stimulated electrically at regular intervals.

In equiactive concentrations procaine, lidocaine and ravocaine® had essentially the same rate of penetration.

Dibucaine was the least penetrant and WIN 3766 the most penetrant of the compounds tested.

Recovery from anesthetic blockade was fairly rapid for procaine, lidocaine and ravocaine®, and much slower for WIN 3766 and dibucaine. The order of recovery was as follows: procaine > lidocaine > ravocaine® > WIN 3766 > dibucaine.

References

4. Luduena, F. P., and Hoppe, J. O.: To be published.

If You Plan to Index Your Anesthetic Machines—This is Important

Arrange for the immediate use of all cylinders in your stocks which are equipped with non-indexed valves. They are useless on indexed equipment. Prevent confusion and avoid hazards in the future. Have only indexed cylinders on hand BEFORE adapters are installed on your equipment.

Most new anesthetic machines are now being delivered equipped with indexed yokes. If you have one on order, the proper rotation of your cylinder stocks should receive prompt attention. Major producers have shipped nothing but indexed cylinders since January 1, 1953. However, you probably still have some “idlers” which may cause trouble later if not used NOW.