Wigglesworth (*3*) has claimed that substances with juvenile hormone activity must be dissolved in oil to reveal their potency. Schneiderman and Gilbert (*4*) have shown that one of the common constituents of vegetable oils (β-sitosterol) has the effect of a juvenile hormone. In a later paper Krishnakumaran and Schneiderman (*12*) found that farnesol (an analog of juvenile hormone) had different effects when dissolved in peanut oil than when injected as an aqueous emulsion. It seems to us that differences such as this are perhaps attributable to synergism between the juvenile hormone (or its analogs) and minor constituents of the oil that may be mimicking the molting hormone or activating the PTG, or which may themselves have a subthreshold juvenilizing effect which is not itself evident in the controls.

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13. Injection of 25 μg of β-sitosterol into newly molted fourth instar *Locusta migratoria* (Reiche & Fairmaire) led to a 3.1 percent increase in nuclear diameter in the PTG; this barely liminal increase gave $P < .05$.

17 September 1968; revised 30 October 1968

Local Anesthetics: Effect of pH on Activity

Abstract. *Lidocaine and dibucaine are more effective in neutral than in alkaline solution when tested on the nonmyelinated fibers of the desheathed vagus nerve of the rabbit. Procaine, however, is more effective in alkaline solution. The activity of benzocaine is unaffected by* p*H. Both the charged and uncharged forms of local anesthetics thus seem capable of blocking conduction.*

Most local anesthetics in common use are secondary or tertiary amines with pK_a values that lie between 8 and 9; in physiological solutions, therefore, they exist both as uncharged and as charged molecules. When such agents are applied to intact tissue, such as the cornea or whole nerve trunks, they are usually more effective in alkaline solution, in which the uncharged form predominates, than in neutral solution, in which the cationic form predominates. This observation led to the belief, generally held until recently, that the uncharged form was the active form of the molecule at the nerve membrane (*1*). However, there is now strong evidence, obtained from experiments on desheathed nerve, for the hypothesis that local anesthetics block conduction in the cationic form, and that they are more effective in alkaline solutions simply because the uncharged form penetrates the tissue barriers more readily (*2–5*). Our experiments indicate that this hypothesis needs to be slightly modified.

We examined the effect of various local anesthetics on the size of the C elevation of the compound action potential of the nonmyelinated fibers of the rabbit vagus nerve. Electrical records were made on the desheathed nerve in the sucrose-gap apparatus, and on the sheathed nerve in a paraffin oil bath (*5*). The temperature of the nerve was 20° to 24°C.

Lidocaine and dibucaine were more effective on the nonmyelinated fibers of desheathed nerves when applied at *p*H 7.2 than at *p*H 9.2 (Fig. 1, A and B), thus confirming the previous experiments that led to the conclusion that the cationic form is responsible for local anesthesia (*6*). However, procaine (Fig. 1 C), which had not been tested before, was more effective at alkaline *p*H, even when tested on the same desheathed vagus nerves in which lidocaine and dibucaine gave the opposite result. Spontaneous hydrolysis of procaine is unlikely to account for this finding, because such hydrolysis would be expected to be more prevalent in alkaline than in neutral solution and would thus lead to a decrease in local anesthetic activity as the *p*H is increased. Anyway, this possibility was excluded by the demonstration that the less active neutral solution of procaine became more active when it was subsequently made more alkaline, whereas the original alkaline solution became less active on being buffered to about *p*H 7. Furthermore, enzymatic hydrolysis was also unlikely to be responsible for the lowered activity in neutral solution, because the addition of an anticholinesterase to the Locke solution (1 m*M* diisopropyl phosphofluoridate) did not affect the rate or degree of block produced by the procaine. One explanation for the anomalous action of procaine might be that the uncharged form of this drug does indeed possess local anesthetic activity. An alternative explanation would be that removal of the external nerve sheath, the epineurium, removes only one of the barriers that retards the diffusion of charged molecules, and that the greater effectiveness of procaine in alkaline solution merely reflects the greater ability of the uncharged form of procaine to penetrate the remaining barrier. Such a barrier is quite likely to exist. Thus Skou (*6*), working with myelinated fibers of frog sciatic nerves from which the epineurium had been removed, found that local anesthetics were more effective in alkaline solution; and Ariëns and Simonis (*3*) have shown that Skou's result can be simply accounted for by postulating that even in the stripped nerve there remains some diffusion barrier to cations between the external medium and the biophase immediately surrounding the membrane. But whatever explanation applies for procaine, only one explanation can apply to the two other anesthetics tested. Whereas the greater effectiveness of procaine in alkaline solution could merely result from a greater penetrability of the uncharged form and would thus be consistent with either the charged or uncharged form being active, the finding with lidocaine and dibucaine can only be explained if the cationic form is active (*1*).

The argument for the cation's, and against the uncharged form's, being active is largely based on experiments with desheathed nerves such as those just described with lidocaine and dibucaine (*1, 5*) and on experiments with single lobster axons (*4*). However, our experiments show that even in sheathed preparations the relative ineffectiveness of the uncharged form can be demon-

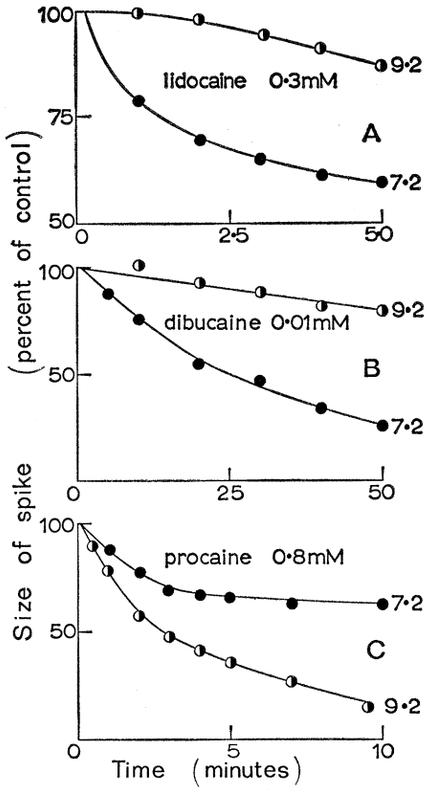

Fig. 1. The effect of lidocaine (0.3 mmole/liter), dibucaine (0.01 mmole/liter), and procaine (0.8 mmole/liter) on the size of the C elevation of the compound action potential of the nonmyelinated fibers of a rabbit desheathed cervical vagus nerve. The anesthetics were applied at *p*H 7.2 (●) and 9.2 (◑).

strated. The argument is as follows. In a relatively alkaline solution (for example, *p*H 8.8), more of the drug in the external solution is in the uncharged form, which relatively easily passes the nerve sheath and other cellular barriers that hold back the charged form. Once past these barriers, however, the anesthetic is in an environment that is less alkaline than the external solution because of tissue buffers. A substantial amount of the anesthetic is therefore converted back to its cationic form, and this would account for the alkaline solution's being more effective than the neutral local anesthetic solution. If the outside solution, however, is made extremely alkaline (for example, *p*H 10.6), the *p*H at the nerve membrane might rise sufficiently to keep most of the local anesthetic that penetrated in the uncharged form. In this case, as Ritchie and Greengard (*1*) have argued on theoretical grounds, a given concentration of anesthetic that was more effective when applied in moderately alkaline solution ought to become less effective when applied in extremely alkaline solutions. Experimentally, this is precisely what is found. On a rabbit vagus nerve whose sheath was left intact, lidocaine (300 μmole/liter) was clearly more effective when applied at *p*H 8.8 than when applied either at *p*H 7.2 or 10.6 (Fig. 2).

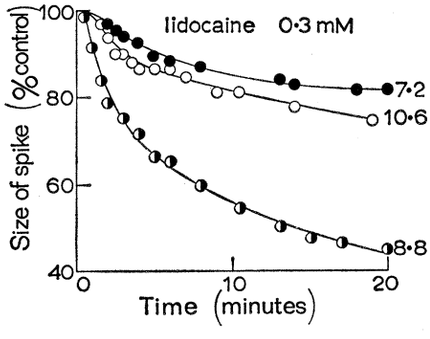

Fig. 2. The effect of lidocaine (0.3 mmole/liter) on the size of the C elevation of the compound action potential of the nonmyelinated fibers of a rabbit cervical vagus nerve whose sheath was intact. The anesthetic was applied at *p*H 7.2 (●), 8.8 (◑), and 10.6 (○).

Our data and those reported previously (*1, 4, 5*) show that the widely held view that local anesthetic activity resides only (or mainly) in the uncharged form of the local anesthetic molecule is untenable. However, it would be unwise to attribute all local anesthetic activity to the cationic form and none to the uncharged form. Benzocaine has exactly the same molecular structure as procaine, except that it lacks the terminal diethylamino group and cannot exist as a substituted ammonium cation. Nevertheless, benzocaine is a potent local anesthetic drug. It is not useful for producing blockage in nerve trunks because of its extremely low solubility. Procaine, for example, is normally applied to intact nerve trunks in a concentration of 50 to 100 mmole/liter, but solutions of benzocaine stronger than 5 mmole/liter are difficult to attain. In desheathed preparations, however, much smaller concentrations of anesthetic are required. Under these circumstances benzocaine is comparable in potency to procaine. In fact, when benzocaine is applied to desheathed vagus nerve at *p*H 7, it is slightly more potent than procaine, although it is less potent that procaine when applied at *p*H 9 (the rate at which the action potential falls being taken as an index of local anesthetic potency). As might be expected (Fig. 3), the local anesthetic activity of benzocaine is independent of *p*H.

Our experiments with lidocaine and dibucaine, together with those reported previously, emphasize the importance of the cation in producing local anesthesia. However, they do not deny local anesthetic activity to the uncharged form. Indeed, the experiments with benzocaine and procaine suggest that both forms of the molecule are active. In the case of procaine, the uncharged form would seem to be relatively more active than the cationic form, whereas with most other local anesthetics (for example, lidocaine, Fig. 2) the charged form is much more active. A plausible interpretation of the findings is therefore: (i) true local anesthetic activity resides in the aromatic lipophilic part of the local anesthetic; and (ii) the presence of a charged cationic head to the molecule intensifies this activity but is not absolutely required.

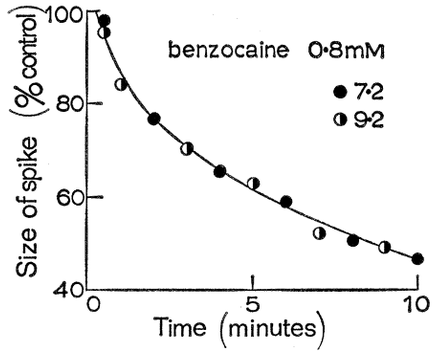

Fig. 3. The effect of benzocaine (0.8 mmole/liter) on the size of the C elevation of the compound action potential of the nonmyelinated fibers of a rabbit desheathed cervical vagus nerve. The benzocaine was applied at *p*H 7.2 (●) and 9.2 (◑).

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16 September 1968; revised 25 October 1968