Insect Hormones: Olive Oil Is Not an Inert Vehicle for Hormone Injection into Locusts

Abstract. *Like other fatty oils from plants, olive and peanut oils, the most commonly used vehicles for the injection of juvenile hormone and its analogs, contain substances that show effects similar to those of this hormone. A dose of olive oil smaller than that used to dissolve a single injection of juvenile hormone will cause nuclear enlargement in the cells of the prothoracic gland of the desert locus and advance the succeeding molt.*

A study on purified juvenile hormone (*1*) leads us to draw attention to a problem in methodology. Assays of fractions containing juvenile hormone activity have usually been carried out with olive oil (*1–3*), peanut (*Arachis*) oil (*4, 5*), or linseed oil (*3*) being used as solvent and diluent. We have found that, when injected into locusts, olive oil affects the locust in a way similar to that of the molting hormone of insects. It is thus probable that test insects have been injected with an analog of the molting hormone (or an analog of the activation hormone from the brain, which stimulates its secretion), as well as with the intended juvenile hormone (methyl-*trans,trans,cis*-10, 11-epoxy-7-ethyl-3, 11-dimethyl-2, 6-tridecadienoate) or its analogs.

The molting hormone of insects is believed to be produced by the prothoracic gland (PTG) and to be an ecdyson (*6, 7*). If a fresh homogenate of this gland is injected into nymphal locusts at an appropriate moment in the stadium, the time of the next molt is significantly advanced (*7*). The amount of the molting hormone produced varies during the stadium and is correlated with changes in the nuclei of the secreting cells of the PTG (*8*). If PTG homogenate is injected into locusts at the time when the nuclei of the gland are just beginning to enlarge (preparatory to an active phase of secretion), this nuclear enlargement is hastened, and the next molt is advanced. The action of PTG homogenate can be simulated by a few other substances, including the plant hormone gibberellic acid A_3 and extracts of plants and of locusts, containing ecdysons or their derivatives (*7*).

We find that purified olive oil (British Pharmacopeia) causes enlargement of the cell nuclei of the PTG when injected into newly molted fourth-instar nymphs of the desert locust *Schistocerca gregaria* (Forskål) (Table 1) and significantly advances the time of the next molt (Fig. 1). As little as 0.4 μl of olive oil produces significant effects; in our few tests with peanut oil we do not observe any effect.

Olive oil, peanut oil, and linseed oil are largely polyunsaturated triglycerides, but all three contain more than traces of sterols and cyclic triterpenoids (*9*). The oils contain β-sitosterol [which possesses some juvenile hormone activity and activates the PTG of diapausing brainless moths (*4*), a result which we have confirmed for locusts (*1*)], stigmasterol and campesterol, and several polycylic triterpenoid alcohols, including α- or β-amirine, cycloartenol, and 24-methylenecycloartenol. Olive oil also contains butyrospermol and several unidentified triterpenoids which have not been found in *Arachis* or linseed oils.

Most investigators of juvenile hormone have injected 1 μl or more of oil. In at least one study, no separate controls of the effect of oil alone were reported (*10*). In another, it was noted in passing that various oils had a local effect on the cuticle (*11*), but in most experiments with juvenile hormone, the authors state that the oil alone had no significant juvenile hormone effect on their test animals (*1–5*). We do not doubt that this is correct, but some attention should be directed to possible interactions of juvenile and molting hormones. The two hormones act upon the same process (molting), the balance between them is crucial for any particular molt, and the juvenile hormone may activate the PTG to secrete the molting hormone (*4*). Diluting juvenile hormone for assay by injection with an oil that contains more than a threshold amount of a substance which produces some of the effects of the molting hormone of the PTG or of the activation hormone of the brain seems to us liable to produce results which are difficult to interpret.

Table 1. Mean diameter (and standard error) of prothoracic gland nuclei 24 hours after injection of substances under test. Each animal was injected with 1.2 μl within 3 hours of the molt to the fourth instar. Ten unfixed, unstained nuclei measured from each of six to nine animals from each group.

Substance injected	Diameter (micron)
Controls	
None	10.97 ± 0.091
Ethanol (50 percent)	10.10 ± .116
Distilled water	10.23 ± .104
Experimentals	
Gibberellic acid A_3	15.07 ± 0.129*
Olive oil	13.57 ± .099*
Peanut oil	10.23 ± .097

* Significant difference from control values ($P < .01$).

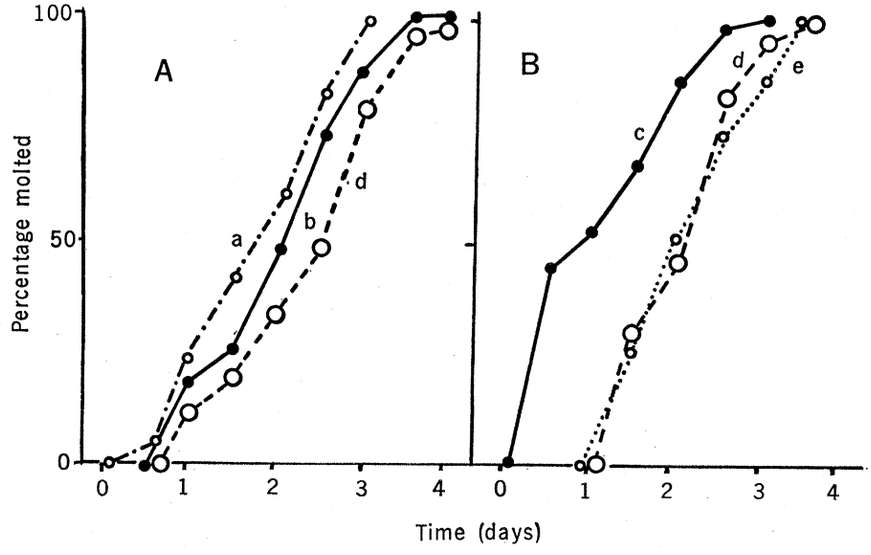

Fig. 1. Time of molt (counting from the time when the first animal molted) for desert locust nymphs which had previously molted within 3 hours of each other and were then injected immediately. In two different experiments (A and B) 12 or more locusts were given each treatment. Curves *a* and *b*, 0.4 μl of different batches of olive oil; curve *c*, 1.2 μl of a third batch of olive oil; curve *d*, 0.4 and 1.2 μl (in experiments A and B respectively) of 50 percent ethanol (the standard solvent in our laboratory for ecdyson injections); curve *e*, 1.2 μl of refined peanut oil (B.P.). Treatment shown in curves *a*, *b*, and *c* gave significantly different results (molting significantly earlier) from *d* and *e* ($P < .05$).

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 $P < .05$.

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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-