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Practical Aspects of Insect Control by Juvenile Hormone

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The development of juvenile hormone (JH) analogues for insect control, and their laboratory and field testing, are now being carried out on an industrial scale. Studies of the hormone action at the cellular level, and of the many implications of JH applications on various stages of the insect life-cycle, are lagging because such studies require long-term scientific involvement. Although many analogues have little specificity for particular insects, the ultimate effects may be very different. Various considerations that may influence the commercial development of JH are discussed.

THE CONCEPT OF CONTROL BY JH

The application of juvenile hormone (JH) in a way that is detrimental to an insect is based on the fact that during the development cycle there are very precisely timed periods during which natural JH is present in or absent from the body fluids and also, perhaps, some of the organs that have a storage function. The coupling of this sequence to an even more rigidly timed sequence of moultings introduces the possibility that any disturbance in the JH level may be irrevocable and ultimately lethal.

So far, we have learned how to apply JH or active analogues successfully to insects only at periods when the natural hormone is absent, or present at low titres. In this way, we can produce intermediate forms in metamorphic moults (larva–adult, larva–pupa, or pupa–adult) or abnormalities in embryogenesis, most of which are ultimately lethal. We have also learned to use these hormones to break the adult reproductive diapause, with the seemingly contradictory result that while a low dose stimulates viable egg production, a higher one also causes the disruption of embryonic development. Although no moult is involved in this case, the results seem to be irreversible, but the reasons are unknown.

Intensive studies of insect endocrinology over many years have shown that from the results of surgical techniques and gland transplantation it is possible to predict nearly all the effects that can be obtained by the application of pure hormones or their analogues, with the exception of effects on embryogenesis. Defining the sensitive periods in different developmental stages for various insects allows for little generalization. Embryogenesis is usually sensitive until blastokinesis occurs, the sensitive period varying from a few hours (in eggs of Coleoptera) to several days (in eggs of Hemiptera). For the metamorphosis from larva to adult, or pupa to adult, the same holds true, the period of sensitivity being usually about a quarter of the duration of the particular instar. Larva–pupa metamorphosis is more complicated; the preceding larval instar has different sensitivity periods for different parts of the body and different organs, and consequently only a long exposure to JH can produce the full effect—i.e., a viable extra larva. However, many exceptions have been found. Diptera cannot produce additional larval instars, as far as we know, and any application of JH in the last larval instar acts, in effect, on the adult metamorphosis and not on pupation. (The pupa itself is no longer sensitive in mosquitos.)

Another interesting phenomenon is the diapause-like condition induced by JH in final instar coleopteran larvae, which seemingly need to dispose of JH before pupation. Although *normal* pupation usually ensues after a long delay, the subsequent adult development is often abnormal. Other species of insect, particularly some large ones, seem to be almost invulnerable to exogenous hormone in the larval stages, examples being the tobacco hornworm (*Manduca sexta*) and the cecropia silkmoth (*Hyalo-*

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phora cecropia), the larva of which does not even respond well to its own authentic hormone (in contrast to the sensitive pupa).

METHODS OF APPLICATION

The finding that purified hormones could easily penetrate insect cuticle came as a surprise; no physiological explanation has yet been given of this fortunate coincidence, as far as we know. It would be wrong to assume that the hormone effectively penetrates into the body cavity in this way, since nearly all the effects are observed in the integument; in the case of diapause breaking, however, penetration is more likely since the gonads are involved. It is known that gland transplants can have profound effects on internal organs, but much more information is needed on the effects of external applications of JH.

Injections of JH have proved to be highly successful when a dilute solution in oil is administered; emulsions, pure hormone, and dilutions in carriers that are miscible with water have always proved to be very ineffective. The " dilution paradox " is a strange but illuminating phenomenon. An increase of effect is obtained by the injection of the same amount of JH in a larger volume of oil. These and other results have led to the conviction that continuous exposure to low doses is much more effective than the application of large single doses. Part of the explanation is to be found in recently completed studies that indicate that injected cecropia JH, which is an ester, is degraded to the inactive acid and the inactive diol with surprising speed, the time being measured only in minutes. This observation raises questions about the way in which an insect protects its endogenous hormone; possibly the JH is bound to lipoproteins or other carriers. Injections are not, as a rule, substantially more effective than topical applications. Studies with labelled JH have revealed very rapid penetration of the cuticle, which may act as a sink and release mechanism to the underlying epidermis. Even this type of application, however, is not as effective as the use of a contact substrate such as cellulose fibres, or as vapour treatment. Paper can bind JH and release enough on contact to produce effects, but at the same time the substrate protects the bulk of the compound against metabolic and other degradation processes. We must therefore conclude that a slow-release formulation is probably the most suitable way of applying active JH compounds.

RESPONSE TO JH TREATMENT

The overall effects of JH application may be very diverse. The result on eggs is usually simple; if JH is applied early enough, the development is abnormal and emergence does not take place. With slightly lower doses, embryos may emerge but they succumb later. It has also been observed in *Dermestes* that the first-instar larvae may have defective hair coats but they later moult successfully to second-instar larvae. Dosages of JH may sometimes be as low as 0.001 μg per egg for a 50% lethal effect, but in other species the dosage required to produce similar effects is 0.1 μg.

In Diptera, no effect on embryogenesis has been observed so far by direct application of JH to eggs, but female houseflies, as well as female *Aedes* mosquitos, produce nonviable eggs after topical treatments with *high* doses of JH. Sterilization of adult females has been achieved in other groups of insects, but the doses required are usually high, particularly in Lepidoptera. Sterilization by JH does not mean decreased fecundity and males are not affected by the hormone.

Metamorphic effects are extremely varied. Mosaic intermediates are very common, and most of them have great difficulty in moulting, but even if they moult spontaneously, they succumb later; the reasons for this are not always clear but it is possible that the mouth-parts or the mobility may be affected. Bioassays can be assessed by scoring either (*a*) retention of juvenile characters or (*b*) emergence from the moult when pupae are treated. The latter procedure is often sufficiently accurate and is commonly used for flies and mosquitos that show few or no true signs of juvenile retention, except for failure of the male genitalia to rotate normally. When food is treated, or when large doses are applied early in the last larval instar, additional larval instars may develop in some pest species, thereby extending the period during which damage occurs, but only rarely do viable adults emerge. Pupae treated with high dosages moult to pupae again, a process that may be repeated several times without the pupae ever being able to free itself from the earlier pupal cuticles.

The practical difficulties involved in the bioassay of JH activity clearly emerge from the above outline, and rigid standardization of the assay animals is required if quantitative and reproducible results are to be obtained. The 50% inhibition dose (ID_{50}) is misleadingly high, because it applies only to a very short period in the life cycle, but this difficulty may be partly offset by the fact that this assay method neglects many other phenomena that may become apparent only after several weeks. Since the quickest quantitative bioassay requires a week of intensive work, the difficulties involved in assays on reproduction and programming, etc., are evident.

FACTORS INVOLVED IN SUCCESSFUL PRODUCT DEVELOPMENT

So far, most quantitative work has undoubtedly been devoted to the development of proprietary analogues. The number of active JH analogues is very great and the enormous costs of developing agricultural chemicals can be justified only for protected proprietary compounds. The natural hormone has a number of undesirable properties, the double bonds and the epoxide function being vulnerable to photo-degradation. Synthetic hormones can be freed of such properties and at the same time the activity may be improved. Many of these objectives have been partially fulfilled, and the search continues. Several compounds that are considerably more active than the cecropia hormone for certain groups of insects have already been found. Since no other natural insect juvenile hormones have yet been identified, owing to the lack of good sources, we do not know whether the analogues have features in common with other natural hormones. However, some of the analogues are more effective, even on the cecropia moth.

The practical value of a chemical for insect control cannot as a rule be derived from laboratory experiments alone, and insect hormones are no exception to this rule. Until a year ago, insufficient materials were available for field tests, but since then some experiments (largely with negative results) have been carried out on field crops such as cotton. We feel that the poor results were caused mainly by the harsh environmental conditions (especially the bright sunlight) under which the trials were made, the inherent instability of the compounds available at present, the difficulty of studying long-term effects in small plots or cages, and the asynchronous characteristics of the insect populations. Suitable formulations may alleviate some of these problems but in the meantime other more suitable targets may present themselves, such as insects in environments that are reasonably well protected against climatic conditions.

For applications in more exposed situations, the most immediate attention should perhaps be given to insects with relatively synchronous populations and to populations of insects with very short life-cycles. Mosquitos seem to be a good target because the larvae cause no damage to crops and the aqueous environment may afford some protection to the compound used as a larvicide. For mosquitos, effectiveness of JH compares favourably with that of several insecticides in use (ID_{50} 0.01 ppm on the last larval instar). Research on embryogenesis and reproduction of insects may yield more useful information on ways to attack pest species.

The industrial development of any compound at the present time involves several other factors. The specificity of a compound for limited groups of insects cannot be expected to raise much industrial enthusiasm, considering the development costs for a single product. Speculation about the development of resistance to JH analogues is premature, since experience has taught that this phenomenon can be studied only as it develops under actual field conditions; no useful contributions to the subject can be made at this time.

Research into all the possibilities of these new hormone alternatives to insecticides has only just begun; studies on reproduction, programming of development and behavioural characteristics, induction of polymorphis, etc., are most needed. The possibility of inducing negative feedback on the JH-producing system by JH application seems theoretically feasible and would be devastating to an insect population (the possibility has already been demonstrated by transplantation and subsequent removal of corpora allata). JH-antagonistic compounds may also be discovered. It is necessary that all levels of development and research should be started at once because even if an ideal compound is soon discovered, it will take several years for commercial production to be realized.

DISCUSSION

BOWERS: Have you been able to obtain contagious sterility in insects other than *Pyrrhocoris*?

STAAL: Contagious sterility has never been demonstrated for insects other than *Pyrrhocoris*, which is particularly sensitive and for which particularly effective analogues are known. It is not impossible that some effects may be seen on other insects with the most active available analogues, but the necessary conditions will be difficult to attain.

FUKUTO: Are any of the juvenile-hormone-type compounds active against insects by ingestion?

STAAL: The most active juvenile-hormone analogues can be very effective when applied in the food. In some cases, concentrations as low as 0.1 ppm can be effective. It is particularly difficult, if not impossible, to ascertain whether the principal route of entrance is ingestion or penetration through the exoskeleton.