INHIBITION OF A JUVENILE HORMONE ANALOGUE BY ORGANIC ACIDS IN TESTS WITH MUSCA DOMESTICA L. AND ONCOPELTUS FASCIATUS (DALLAS)

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ABSTRACT

INHIBITION OF A JUVENILE HORMONE ANALOGUE BY ORGANIC ACIDS IN TESTS WITH MUSCA DOMESTICA L. AND ONCOPELTUS FASCIATUS (DALLAS)

By

Thomas M. Brown

The "juvenile hormone effects" of 10,11-epoxy farnesenic acid methyl ester on the housefly were inhibited by separate application of citric acid, tricarballylic acid, α-ketoglutaric acid, malic acid, oxalacetic acid, or succinic acid. With the exception of succinic acid, these acids also inhibited the action of the hormone analogue in assays on the milkweed bug. Opposite-site applications, thin-layer chromatography, and gas-liquid chromatography showed that the inhibition was due to a chemical reaction, possibly cyclization of the hormone analogue either on or in the insect cuticle.

INHIBITION OF A JUVENILE HORMONE ANALOGUE BY ORGANIC ACIDS IN TESTS WITH *MUSCA DOMESTICA* L. AND *ONCOPELTUS FASCIATUS* (DALLAS)

By

Thomas M. Miller Brown

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INTRODUCTION

Herzog and Monroe (1971) found that housefly pupae, Musca domestica L., were susceptible to treatment with 10,11-epoxy farnesenic acid methyl ester, and they also noted that citric acid had an inhibitory effect in synthetic juvenile hormone pre-treated flies. Too, Grassman et al. (1968) showed that farnesyl methyl ester injections were inhibited by certain pupal fat extracts in yellow mealworm pupae, Tenebrio molitor L.

In these studies, the housefly and the milkweed bug, Oncopeltus fasciatus (Dallas), were used to study the effects of different di- and tricarboxylic acids, including Kreb's cycle intermediates, on the action of a synthetic juvenile hormone analog, and attempts to study the mechanisms of the observed effects were made.

MATERIALS AND METHODS

Experimental animals

The insects used in these studies were the housefly, Musca domestica L., and the large milkweed bug, Oncopeltus fasciatus (Dallas); the latter was used as an assay insect for juvenile hormone active compounds (Bowers, 1968). The houseflies were an insecticide-susceptible, maximum longevity strain in which larvae were routinely reared on CSMA medium (Anonymous, 1959), and the adults fed a 1:1 mixture of dry, nonfat milk and sucrose. The milkweed bugs were reared in glass jars on milkweed seed. Cultures were maintained at 26-27°C and 50% RH. For testing, housefly eggs were collected, surface sterilized with hypochlorite solution and transferred to aseptic synthetic diets (Monroe, 1962). When the larvae were fully developed and migrating, they were washed from the diets and placed in Petri dishes. White pupae were collected and dated as zero hours old. Only pupae 2-5 hours old were treated.

Milkweed bug eggs less than one day old were collected and placed in glass rearing jars with milkweed

seed and water. The first fifth instar bugs to appear were removed and discarded, and test insects were collected the following day so that all were fifth instar nymphs less than 24 hours old.

Experimental chemicals

The synthetic juvenile hormone (SJH) used in these studies was 10,11-epoxy farnesenic acid methyl ester synthesized according to Bowers et al. (1965). Glass distilled acetone was used as the solvent for all chemicals except fumaric acid which required an acetone-water (10:1) mixture for solubilization. All solutions were freshly prepared for each series of tests.

Treatment techniques

All treatments were made with a calibrated microapplicator (Biotronics, Brookings, South Dakota). In treating the housefly pupae, 10 µg of SJH was applied topically in 1 µl of acetone. After evaporation of the solvent, 20 µg of a candidate organic acid was administered topically in 1 µl of acetone.

Milkweed bugs were treated with 5 µg SJH applied topically in 1 µl of acetone to the abdomen. After evaporation of the solvent, 10 µg of a candidate organic acid was applied in 1 µl of acetone to the same site, or in certain tests, to an opposite site on the abdomen.

Control insects were treated with 1 µl of acetone. SJH-treated insects which did not receive acid treatment received an additional 1 µl of acetone. Each test group included 25 insects.

Evaluation of tests

All test insects were allowed to develop for 8 days and then evaluated. Houseflies were scored as normal adults (emerged flies, morphologically normal), abnormal adults (flies partially emerged from puparia or with abnormal wings), and non-emerged flies (flies which did not emerge, but when dissected possessed both adult and pupal characteristics).

Milkweed bugs were evaluated as normal adults morphologically normal), abnormal adults (bugs with adult shape and coloration patterns, but with abnormal wings or thorax), supernumerary nymphs (bugs which moulted, but had nymphal shape, coloration and wing pads) and fifth instar nymphs (bugs which did not moult).

Thin-layer chromatography

SJH was chromatographed by thin-layer chromatography (TLC) with and without the organic acids on an aluminum oxide G thin-layer plate developed in chloroform-ethyl acetate (2:1). The spots were visualized with phosphomolybdic acid. SJH was also chromatographed with

and without organic acids on silica gel G thin-layer plates developed in chloroform-pentane (2:1) and visualized using iodine vapors.

Gas-liquid chromatography

SJH with and without organic acids was analyzed by gas-liquid chromatography (GLC) using a gas-liquid chromatograph equipped with dual hydrogen flame detectors (Research Specialties Corp., Series 600). A 2 m glass column was packed with 100/120 mesh Gas Chrom Q coated with 3% XE-60 by means of a Hi-Eff Fluidizer (Applied Science Laboratories, State College, Pennsylvania). The column temperature was maintained at 152°C.

Standard solutions of SJH in acetone and SJH in ethanol were prepared and chromatographed. Acetone solutions of equimolar amounts of SJH and acid were prepared and chromatographed for each acid studied. The chromatograms of the SJH + acid solutions were compared to the chromatograms of the standard SJH solutions.

RESULTS

Figure 1 and Table 1 summarize the results of tests with the housefly. SJH-treated flies, when compared to control flies, showed a 22% increase in non-emerged flies, a 23% increase in abnormal adult flies and a 45% decrease in normal adult flies. However, when α-ketoglutaric acid was applied after SJH-treatment, SJH increased non-emerged flies only 4%, increased abnormal adult flies 1.6% and decreased normal adult flies 4% from the controls. This inhibition of SJH activity in the housefly was also observed to lesser degrees with citric acid, tricarballylic acid, malic acid, oxalacetic acid, and succinic acid. One test with fumaric acid demonstrated no inhibitory effect on SJH.

Figure 2 and Table 2 summarize the results of tests with the milkweed bug. Treatment with SJH produced 62% supernumerary nymphs, and normal adult bugs were reduced from 90% in the controls to 0.8% in the SJH treated group. SJH-treated bugs also showed an increase in abnormal adults and an increase in fifth instar nymphs over the controls.

TABLE 1. Topical Application of 10,11-Epoxy Farnesenic Acid Methyl Ester (SJH) With and Without Organic Acids on Housefly Pupae 2-5 Hours of Age.

Test group	No. of tests	Non-emerged flies	Abnormal adults	Normal adults
Control (acetone)	5	10.4	2.4	87.2
SJH	4	32.0	25.6	42.4
SJH + citric acid[a]	5	25.6	11.2	63.2
SJH + tricarballylic acid[b]	2	12.0	8.0	80.0
SJH + α-ketoglutaric acid[c]	3	14.7	4.0	81.3
SJH + malic acid[c]	3	26.7	5.3	68.0
SJH + oxalacetic acid[d]	1	20.0	4.0	76.0
SJH + succinic acid[a]	3	20.0	16.0	64.0
SJH + fumaric acid[a]	1	44.0	12.0	44.0

[a]Acid purchased from Fisher Scientific Company, Fair Lawn, New Jersey.

[b]Acid purchased from Aldrich Chemical Company, Milwaukee, Wisconsin.

[c]Acid purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[d]Acid purchased from Calbiochem, Los Angeles, California.

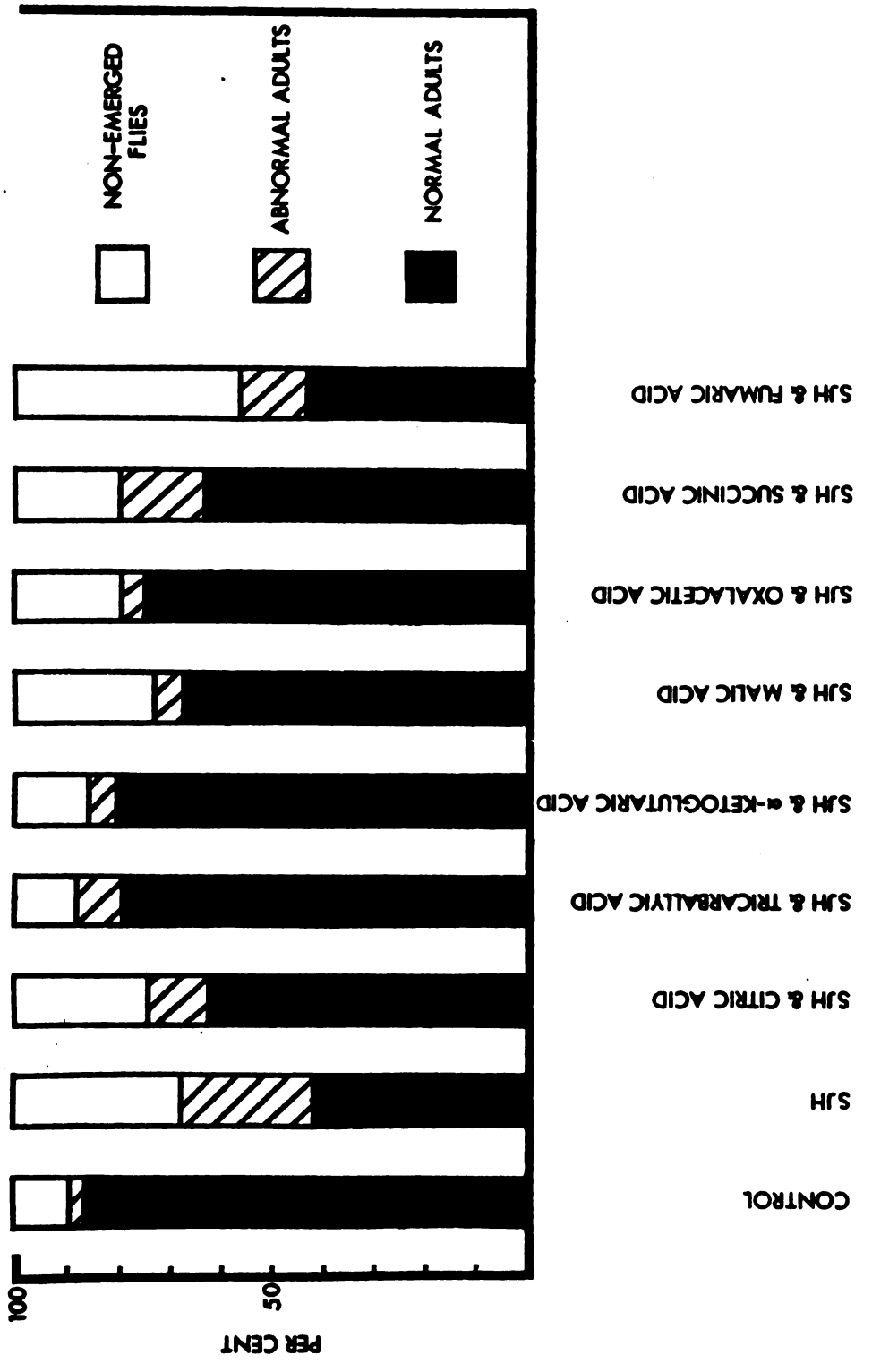

Fig. 1. Inhibitory Action of Organic Acids on 10,11-Epoxy Farnesenic Acid Methyl Ester Demonstrated in Housefly Assays.

TABLE 2. Topical Application of 10,11-Epoxy Farnesenic Acid Methyl Ester (SJH) With and Without Organic Acids on Milkweed Bug Fifth Instar Nymphs.

Test group	No. of tests	% Fifth instar nymphs	% Super-numerary nymphs	% Abnor-mal adults	% Nor-mal adults
Control (acetone)	4	6.0	0.0	4.0	90.0
SJH	6	27.3	62.0	10.0	0.7
SJH + citric acid	4	12.0	7.0	38.0	43.0
SJH + tri-carballylic acid	3	21.7	8.0	29.0	41.3
SJH + α-ketoglutaric acid	4	14.0	7.0	38.0	41.0
SJH + malic acid	4	11.0	17.0	22.0	50.0
SJH + oxal-acetic acid	3	4.0	10.7	21.3	64.0
SJH + succinic acid	4	29.0	54.0	17.0	0.0
SJH + fumaric acid	3	13.3	54.7	26.7	5.3
SJH + citric acid on opposite site	3	26.7	42.7	29.3	1.3

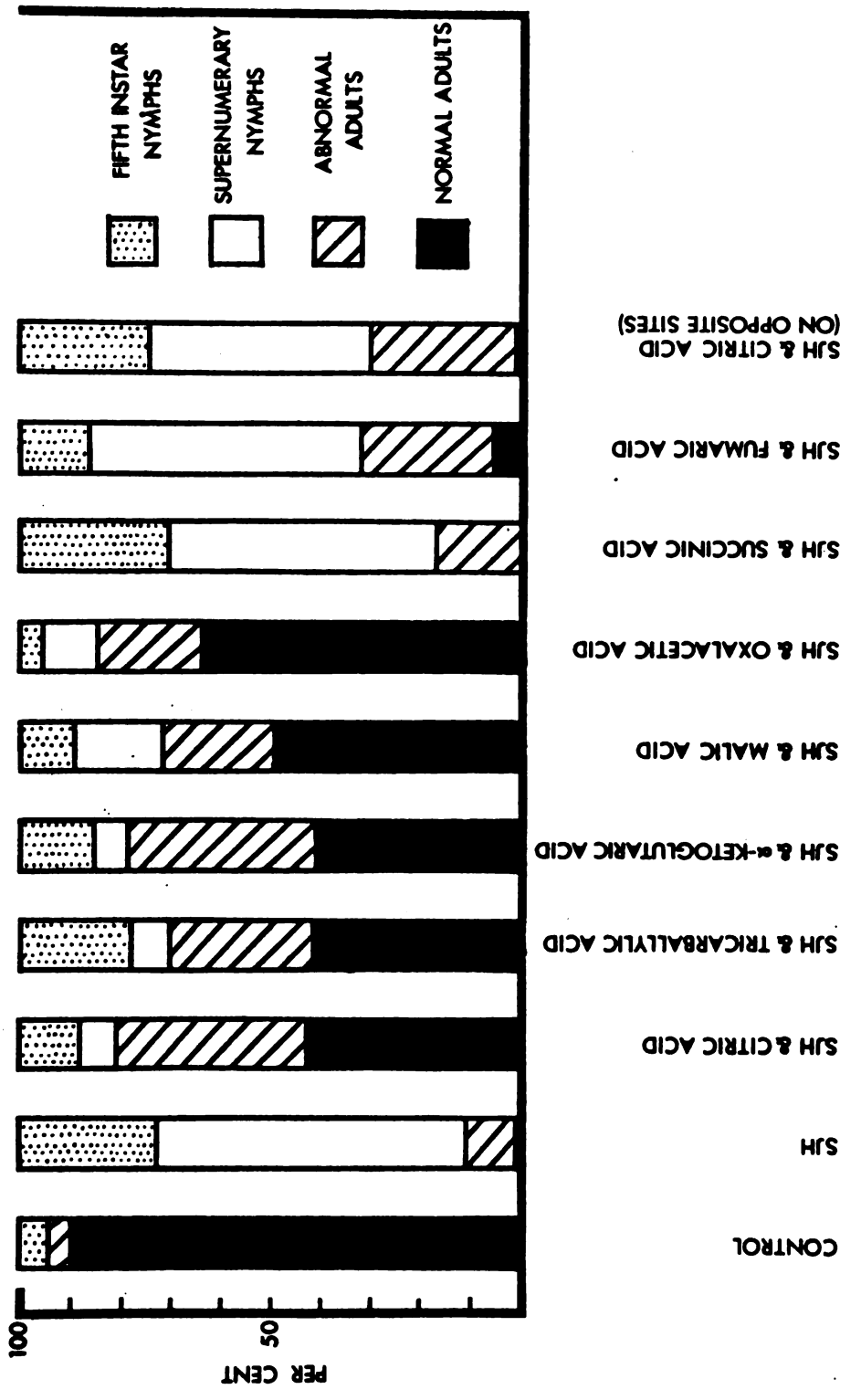

Fig. 2. Inhibitory Action of Organic Acids on 10,11-Epoxy Farnesenic Acid Methyl Ester Demonstrated in Milkweed Bug Assays.

Compared to the bugs treated with SJH alone, bugs which received both hormone and citric acid showed: an increase in normal adults, an increase in abnormal adults, a decrease in supernumerary nymphs, and a decrease in fifth instar nymphs. A significant reduction in supernumerary nymphs (55%) and a significant increase in normal adult bugs (42%) demonstrate inhibition of hormone activity. This inhibition found with citric acid was also observed with tricarballylic acid, α-ketoglutaric acid, malic acid, and oxalacetic acid. Succinic acid and fumaric acid did not exhibit significant inhibition of SJH on the milkweed bug.

When citric acid was post-applied to a site opposite that of the hormone application, normal adult bugs were increased only 0.6% and supernumerary nymphs were reduced only 19% from the SJH-alone-treated group. This represented a much less significant inhibition of the hormone than was found when hormone and acid were applied to the same site. The same phenomenon was observed in preliminary tests with α-ketoglutaric acid.

Thin-layer chromatography showed new spots appearing when SJH and various acids were spotted together and chromatographed. The new spots were most apparent

with α-ketoglutaric acid and oxalacetic acid, less apparent with malic acid, citric acid and tricarballylic acid and least apparent with succinic acid and fumaric acid.

Gas-liquid chromatography demonstrated a marked decrease in the SJH peak with solutions in which citric acid, tricarballylic acid, α-ketoglutaric acid, malic acid, and oxalacetic acid were present in a 1:1 molar ratio with the hormone. In the case of α-ketoglutaric acid, the hormone peak disappeared gradually over 7 injections spaced 24 minutes apart. This marked decrease in the hormone peak was not observed with succinic acid nor with fumaric acid.

SJH in acetone was chromatogramed alone and compared to SJH in ethanol. The chromatograms were identical.

DISCUSSION

These studies confirmed the findings of Herzog and Monroe (1971) by demonstrating that housefly pupae were sensitive to SJH application and that citric acid applied separately to hormone-treated pupae inhibited the action of the hormone. These studies further demonstrated that other organic acids inhibited SJH activity in the housefly. Similar results with the milkweed bug showed this phenomenon was not limited to housefly assays. "Opposite-site" applications on the milkweed bug indicated that direct contact of acid with hormone is required for significant inhibition, and thin-layer and gas-liquid chromatography showed that organic acids could apparently alter SJH chemically. It is believed that the SJH was also chemically changed in the assay tests.

Stork and Brugstahler (1955) noted that farnesoic acid treated with mineral acids underwent a series of cyclization reactions, and Law et al. (1966) found the products of these reactions to have no juvenile hormone activity. Van Tamelen et al. (1966) showed that

10,11-epoxy farnesenic acid methyl ester treated with phosphoric acid gave rise to a bicyclic hydroxy-ester, an acyclic keto-ester, a monocyclic hydroxy-ester and a bridged ether. Trost (1969) investigated the biological activity of the bicyclic hydroxy-ester and the monocyclic hydroxy-ester and found them to be inert. Meyer et al. (1969) mentioned that "cecropia juvenile hormone" lost activity on exposure to 0.3 N perchloric acid in methanol.

It is possible that cyclization occurred with the organic acids found to inhibit SJH in these studies. However, no new peaks appeared in the GLC studies and the new spots found in TLC studies did not chromatogram on the XE-60 GLC column. The products of these reactions will be investigated in future studies.

It must be noted that the effects shown on the insect cuticle (in vivo studies) may be of a different chemical nature than those observed in the chromatographic (in vitro) studies. Since the hormone and the acid were in solution for only a few seconds in the biological tests, the possibility exists that some cuticular component was involved. The fact that succinic acid inhibited hormone action in the housefly, but did not inhibit hormone action in the milkweed bug may be related to differences in the cuticular structures of the insects.

A cell culture medium containing four organic acids was employed by Mitsuhashi and Grace (1970) in their study of cell multiplication rates which included tests with farnesol, and Minks (1967) used malic acid as a substrate in metabolic studies with corpora allata homogenates. In future experiments such as these, it should be considered that the organic acids present may be inhibiting the juvenile hormone activity of the compounds tested and appropriate controls should be employed.

These studies also suggest the possibility of Kreb's cycle intermediates influencing the actual metabolism-deactivation of juvenile hormone in insects; however, such a possibility awaits further investigations in regards to juvenile hormone metabolism in insects.

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APPENDIX

LITERATURE REVIEW

During the last decade, ever increasing research has been done centering on the morphological effects of many "juvenile hormone-type" compounds on insects. Much of this work has been stimulated by the need for a true insecticide; that is, one which would be toxic only to insects. Although many compounds have been found to cause juvenilizing in insects, and volumes of reports have enumerated the striking morphological changes induced; work focusing on the mode of action, biosynthesis and biodeactivation of the hormone has been comparatively negligible.

Comprehensive reviews of the history of juvenile hormone research have been published. The biology of the hormone and its function in relation to other insect endocrines was extensively discussed by Schneiderman and Gilbert (1964). The chemical aspects of juvenile hormone as isolated from the silk moth, Hyalophora cecropia, were reviewed by Roller and Dahm (1968). Williams (1967) coined the term "third-generation pesticides" to describe juvenile hormone and its analogues in his examination of

the insecticidal potentialities of these agents. Excellent general summaries of juvenile hormone research included an address by Berkoff (1970), an article by El-Ibrashy (1970), and a book by Wigglesworth (1970).

Chemistry

Roller and Dahm (1968) published the structure of juvenile hormone isolated from the cecropia silk moth as determined by mass spectrometry, nuclear magnetic resonance spectrometry, gas-liquid chromatography, and identification of reaction products. Synthesis of the hormone, methyl trans,trans,cis-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, was also described. Meyer et al. (1970) showed that there were actually two cecropia juvenile hormones, one being the tridecadienoate molecule identified by Roller and Dahm, and the other being a dodecadienoate molecule otherwise identical. Dahm and Roller (1970) then found these two hormones to be present in the giant silk moth, Hyalophora gloveri.

Functions

Juvenile hormone is secreted from the corpora allata into the haemolymph in which it is carried to target tissues where it effects a response. Although for many years the juvenile hormone has been claimed to be secreted from the corpora allata, two minute glands

posterad of the insect brain, it was not until the past year that Roller and Dahm (1970) actually isolated the hormone as produced by corpora allata in vitro.

Several functions have been claimed for juvenile hormone, but the most investigated property is its morphogenetic activity. Various viewpoints have been taken as to how growth and differentiation are affected by the juvenile hormone. Williams (1967) suggested that the moulting hormone, ecdysone, actively promotes growth and differentiation toward maturity while juvenile hormone functions as a "brake" or restraint on this development. Roller and Dahm (1968) concurred with this view stating that ecdysone induces metamorphosis and initiates moulting while the juvenile hormone modifies the expression of the moult in such a way as to favor development of larval structures. Wigglesworth (1970) saw a slightly more active role for juvenile hormone by maintaining that it is not only arrests differentiation, but that it has a qualitative effect on growth, possibly by activation of the larval genome. In any case, it has been established that larval moults are accompanied by a high juvenile hormone titer; that this titer declines to allow a larval-pupal moult, while juvenile hormone must be absent during the pupal-adult moult for normal metamorphosis. Wigglesworth (1970) cited that the epidermal cells

involved in certain moults could also be induced to form "younger" cuticle when juvenile hormone was present in greater concentrations than normal. This reversal in "differentiation" supports the hypothesis of an active role for juvenile hormone.

In the adult insect, the juvenile hormone has been shown to produce gonadotropic effects by Roller and Dahm (1968). They used the natural juvenile hormone of cecropia to induce yolk deposition in allatectomized cockroaches, Periplaneta americana and Leucophaea maderae.

Adams and Nelson (1969) discussed the relationship of the corpus allatum to reproduction in the housefly, Musca domestica. They hypothesized that juvenile hormone stimulates vitellogenesis and that an oostatic hormone can inhibit the juvenile hormone when necessary in the reproductive cycle. Adams (1970) found that injection of extracts containing the oostatic hormone caused ovariectomized female houseflies to store juvenile hormone as determined by observation of the corpus allatum.

Sahota et al. (1970) reported that the Douglas-fir beetle, Dendroctonus pseudotsugae, might postpone ovarial development when not on host logs through the withholding of its juvenile hormone.

In similar studies, juvenile hormone has also been implicated as a regulatory factor in diapause.

Wigglesworth (1970) examined this role which has been established through experiments in which brainless, diapausing pupae of various insects have been caused to terminate diapause by injection of hormone analogues. This experimental phenomenon has been termed the prothoracatropic effect by some authors. To investigate this possible regulatory mechanism in nature, de Wilde (1969) used pure juvenile hormone to calibrate his bioassay technique, and then determined the hormone titer of the haemolymph of the Colorado potato beetle, Leptinotarsa decemlineata, through adult life. He found a definite correlation between the juvenile titer and behavior leading to diapause.

Metabolic effects

The effects of juvenile hormone on various metabolic processes in the adult insect have been studied, usually with some attempt to relate the results to either reproduction or diapause. Slama (1964) employed the Warburg respirometer to study metabolism in allatectomized female adult bugs, Pyrrhocoris apterus. He concluded that juvenile hormone regulated reproductive respiratory metabolism. However, in studies of the African migratory locust, Locusta migratoria, Minks (1967) measured respiration and oxidative phosphorylation in

isolated flight muscle mitochondria with α-glycerophosphate and pyruvate/malate substrates, then added homogenized corpora allata. No effect on oxygen consumption was seen with either substrate, but stimulation of oxidative phosphorylation *in vitro* was observed with α-glycerophosphate.

Zalokar (1968), working with the German cockroach, *Blattela germanica*, demonstrated with radioactive precursors that activation of corpora allata (by ootheca removal) stimulated incorporation of uridine-^{3}H into RNA followed by increased protein synthesis.

Induction of yolk protein synthesis by topical application of a juvenile hormone analogue was observed by Brookes (1969) who used the amputated abdomena of adult female cockroaches, *Leucophaea maderae*. Studies *in vitro* with adult female *L. maderae* by Gilbert (1967) showed that lipid synthesis in ovaries was enhanced during oogenesis, while being simultaneously depressed in fat body.

Shaaya and Sekeris (1970) investigated the control of ootheca formation in *Periplaneta americana* finding that juvenile hormone regulated the synthesis of protocatechuic acid.

In a study of adult *Drosophila melanogaster*, Butterworth and Bodenstein (1969) found female corpora

allata and synthetic juvenile hormone to stimulate adipose tissue in the male fly. A controversial result of this study was that simultaneous implantation of both male and female corpora allata did not give the stimulatory reaction.

Metabolic effects of juvenile hormone in developing insect larvae and pupae have also been investigated. Sehnal (1966), who performed implantations of glands with larvae and pupae of the wax moth, Galleria mellonella, concluded that the additional juvenile hormone elicited increased oxygen consumption only when induced morphological changes were taking place. This was in concordance with the concept of indirect metabolic action of the hormone as discussed by Wigglesworth (1970).

Similar results were observed regarding the effect of juvenile hormone on the haemolymph protein of the silk moth, Antheraea polyphemus, by Blumenfeld and Schneiderman (1968). The accumulation of protein in the blood on injection of juvenile hormone extract into female pupae was caused, not by regulation of protein synthesis, but by failure of affected oocytes to utilize this accumulating protein.

On the other hand, juvenile hormone has been shown to have a direct effect on lipid metabolism in developing insects. In studies with Hyalophora cecropia, Stephen

and Gilbert (1970) observed that a high titer was accompanied by a low synthesis of fatty acids. When the corpora allata were inactive in last instar larvae, the lipid was rapidly accumulated. Increasing juvenile hormone titer in pupae corresponded with a decreasing rate of lipid synthesis. They also mentioned that this correlation was found in studies *in vitro*.

Mode of action

It is clearly remarkable that this compound of great biological activity, eliciting spectacular changes in insects, can be so well known in its identity and effects and yet can be completely mysterious in its mode of action. The solving of this mystery awaits further research along the lines of the following pioneering experiments.

Beerman and Clever (1964) noted that injections of ecdysone into the midge, *Chironomus tentans*, brought about explosions or "puffs" of the giant polytene chromosomes of the salivary glands. These puffs were thought to be related to increased gene activity which resulted in increased RNA synthesis. Lezzi and Gilbert (1969), again working with *C. tentans*, reported that ecdysone also affected the Balbiani ring 1 which is a giant puff of a tissue-specific nature. They further showed that juvenile

hormone decreased Balbiani ring 1, but that it induced a puff in another region. From these and additional data, they suggested that juvenile hormone may be an antagonist to ecdysone. Laufer and Holt (1970) working with the midge, Chironomus thummi, and using a mixture of juvenile hormone analogues, also found effects on a Balbiani ring. However, they found no antagonism in relation to the ecdysone puffs in this species.

Although these reports showed that ecdysone and juvenile hormone treatments induced chromosome puffs in vivo, it is not known if this activation was a direct or an indirect result of the hormone. Treatment in vitro to isolated chromosomes in a strictly defined medium must be done to answer this problem.

Chromosome puffs are also caused by changes in metallic ion concentration and the possibility exists that hormones may act indirectly on gene activity through the regulation of cellular ions as discussed by Lezzi and Gilbert (1970). There may even be a longer chain of events initiated by the interaction of the hormone with a lipid membrane as Baumann (1968) has shown juvenile hormone to depolarize salivary gland cell membrane in Galleria mellonella.

Structural analogues and insect control

Control of insects through the disruption of the hormonal system is becoming a possible alternative to the use of persistent pesticides. A very active area of research involves the induction of juvenile hormone effects by synthetic analogues of the hormone.

Two of the most tested compounds are methyl 7,11-dichloro-3,7,11-trimethyl-2-dodecenoate and 10,11-epoxy farnesenic acid methyl ester. The former was contained in the mixed farnesenic acid derivatives prepared according to Law et al. (1966). Insecticidal potentiality of this compound has been demonstrated by Spielman and Skaff (1967) with the mosquito, Aedes aegypti, Vinson and Williams (1967) with the body louse, Pediculus humanus, Thomas and Bhatnagar-Thomas (1968) with pests of stored grain, White (1968) with the cabbage aphid, Brevicoryne brassicae, and Hintze (1969) with Cerura vinula, a moth. This analogue exhibited ovicidal properties on the spruce budworm, Choristoneura fumiferana, in tests by Retnakaran (1970) and on the bugs, Pyrrhocoris apterus and Oncopeltus fasciatus, and the silk moth, Hyalophora cecropia, in experiments by Riddiford (1970a, 1970b).

Bowers et al. (1965) synthesized 10,11-epoxy farnesenic acid methyl ester finding it to be active in

the milkweed bug, Oncopeltus fasciatus, and in the yellow mealworm, Tenebrio molitor. This analogue also produced juvenilizing effects in the flesh fly, Sarcophaga bullata, as shown by Srivastava and Gilbert (1969) and in Musca domestica as demonstrated by Herzog and Monroe (1971).

Hundreds of other natural and synthetic compounds have been found to have juvenilizing activity in various insects. Levinson (1966) found farnesol and nerolidol to be active in Tenebrio molitor. Activity of p-1,5-dimethylhexyl benzoic acid derivatives was noted by Suchy et al. (1968) with bugs of the genus Dysdercus. Bowers (1968) found activity with several well-known insecticide synergists. He also (1969) increased the potency of certain synergists by altering side chains. Wigglesworth (1969) tested forty-two compounds on Rhodnius prolixus. Fifteen analogues were assayed on Galleria mellonella by Jarolim et al. (1969). Combinations of several functional groups and terpenoid skeletons were made and tested on T. molitor by Swartz et al. (1970). Several commercial compounds were assayed in the stable fly, Stomoxys calcitrans, by Wright (1970) and in the greasy cutworm, Agrotis ypsilon, by El-Ibrashy and Mansour (1970). Riddiford et al. (1971) synthesized imino analogues which were observed to exert a

synergistic effect when tested with cecropia juvenile hormone on Antheraea polyphemus and Pyrrhocoris apterus.

Many other compounds have been and are being examined for hormone-mimicking characteristics. Other insects are also being investigated for susceptibility to hormone analogues. There is a need to find the actual structure of juvenile hormone in many more insects, since it is presently known in only one genus. This would be of value in developing more specificity in future insecticides. Elucidation of the biosynthetic and biodeactivation mechanisms of juvenile hormone would also be a great contribution to this developing area. Finally, the determination of the exact mode of action of juvenile hormone would be an exciting contribution to biological understanding.

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