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Synthesis and Transport of Juvenile Hormones in Insects

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Juvenile hormones are potent regulators of certain essential processes in the life cycle of the insect. Two well-defined functions are the "status quo" effect in juvenile insect forms and the promotion of the maturation of the reproductive system in the adult, especially in the female.[2]

Insects are generally divided into two types: those that have a complete metamorphosis, with juvenile forms differing markedly from the adult forms (e.g., caterpillar–pupa–moth); and those that have an incomplete metamorphosis, with juvenile forms resembling adult forms, except for minor differences such as the presence of wings (e.g., grasshopper nymph–grasshopper adult).

The "status quo" effect of juvenile hormone is best illustrated in the case of an animal with complete metamorphosis. The caterpillar (larva) grows and sheds its outer coat several times, but in each case the result is another caterpillar of the same general form. At metamorphosis, shedding of the outer coat leads to a new form, the pupa. In order to maintain larval form, juvenile hormone is necessary. For metamorphosis to proceed, the concentration of juvenile hormone in the blood (hemolymph) must fall to a low level during a certain critical stage prior to shedding of the skin. Thus juvenile hormone in the immature insect exerts an inhibitory effect on the formation of typical adult structures (e.g., wings).

In the adult female, juvenile hormone levels again rise and the hormone now has a stimulatory effect on some aspect of maturation of the reproductive system. The exact process that is stimulated varies with the insect species. In some cases production of egg proteins is stimulated, while in others, deposition of the protein into the eggs is stimulated, but in each case, juvenile hormone is essential to the reproductive function.[2]

Juvenile hormones are produced by the corpora allata, a pair of small glands (volume is usually a few thousand cubic microns) lying behind the brain (Figure 1). A number of experiments show that removal of the corpora allata (allatectomy) in young larvae leads to precocious development of small adults, or in female pupae leads to sterile adults. The effects of allatectomy are reversed by administration of juvenile hormone. In many cases, corpora allata continue to function when implanted into other insects, or when cultured in vitro.[2]

The fact that juvenile hormone must be present at some stages in the life of the insect and absent in others demonstrates that hormone levels must be under some sort of control. It is now evident that hormone levels reflect a balance between synthesis on one hand and uptake and degradation in the tissues on the other.[3]

The chemistry of insect juvenile hormones is now well understood,[4] and the focus of attention has turned to biosynthesis, transport, mechanism of action, and practical uses of these materials. It has been recognized that utility of juvenile hormones and analogues for insect control is limited by a number of factors, including their stability in the environment and their restricted spectrum of action against certain groups of insects and within brief periods of sensitivity.[5] An understanding of the ways in which specific insect tissues or cells interact with or metabolize juvenile hormones may provide insights into new ways for interfering with hormone production or utilization and may ultimately lead to a variety of useful insect control techniques.

We will focus here on what has been learned about hormone biosynthesis and its control as well as hormone interactions in insect blood during the course of its transport to target tissues. It is important to note that the number of insect species is exceedingly large, showing much variation in form and life style. Large variations are found even in metabolic processes, which may vary sharply from one species to another. While

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(2) (a) L. M. Riddiford and J. W. Truman in "Insect Biochemistry", M. Rockstein, Ed., Academic Press, New York, 1978, p 368; (b) W. W. Doane in "Developmental Systems: Insects", Vol. 2, S. J. Counce and C. H. Waddington, Eds., Academic Press, New York, 1973, p 291; (c) F. Engelmann, "The Physiology of Insect Reproduction", Pergamon Press, New York, 1970, p 143.

(3) Y. Akamatsu, P. E. Dunn, F. J. Kêzdy, K. J. Kramer, J. H. Law, D. Reibstein, and L. L. Sanburg in "Control Mechanisms in Development", R. H. Meints and E. Davies, Eds., Plenum Press, New York, 1975, p 123.

(4) B. M. Trost, *Acc. Chem. Res.*, 3, 120 (1970).

(5) G. B. Staal, *Pontif. Acad. Sci. Scr. Varia*, 41, 354 (1976).

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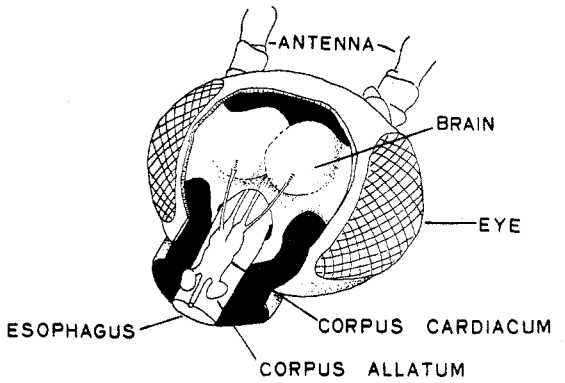
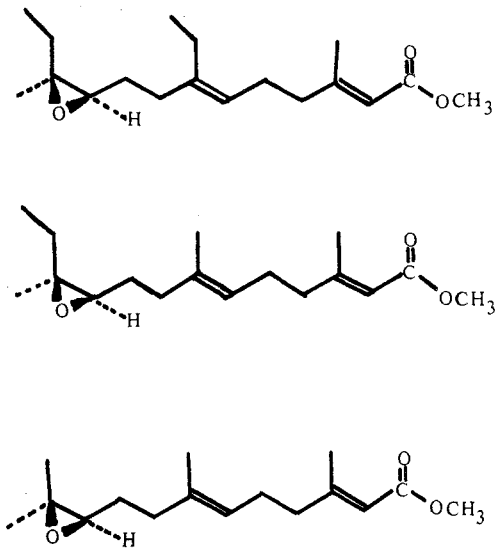


Figure 1. Diagram of the head of an insect with a portion of the cuticle cut away to show the brain and associated organs (redrawn from Engelmann,[2c] with permission; copyright 1973 Pergamon Press).

Scheme I[a]



[a] Top, juvenile hormone I; middle, juvenile hormone II; bottom, juvenile hormone III.

we will discuss work mainly with two insect species, *Manduca sexta*, the tobacco hornworm, and *Leptinotarsa decemlineata*, the Colorado potato beetle, it may be expected that other species may deal with juvenile hormones in very different ways.

Biosynthesis of Juvenile Hormone

The structures of the three naturally occuring juvenile hormones are shown in Scheme I. The simplest compound has a sesquiterpene structure derived from acetate by way of the intermediates mevalonate and farnesyl pyrophosphate. Hydrolytic cleavage to farnesol would then be followed by oxidation of the allylic alcohol to a carboxylic acid and introduction of the methyl ester function and the epoxide group; all of these processes have ample analogies in other biosynthetic systems.

Less obvious is the origin of the "extra" carbons of the two higher homologues, which bear ethyl side chains replacing methyls of the normal sesquiterpene structures. When biosynthetic experiments began, the prevailing idea was that carbon alkylation reactions involving the methyl group of methionine (in its activated form, *S*-adenosylmethionine) might be responsible for the ethyl side chains, in analogy to the formation of ethyl side chains in the plant sterols.[2] It was shown, however, that when labeled methionine was administered to intact animals, label was incorporated exclusively into the methyl group of the ester function and not at all into the ethyl side chains.[6] This discovery was of particular importance, for it introduced the use of isotopically labeled methionine as a valuable precursor for all juvenile hormones—one that is not effectively incorporated into any other lipid-soluble material in the insect.

Attention then turned to the other possibility, namely, that incorporation of propionate[7] in place of acetate could give rise to the ethyl branches.[7a] This was demonstrated most convincingly by Peter and Dahm,[8] who administered [1-^{14}C]propionate to moth pupae (*Hyalophora cecropia*) and isolated and degraded the labeled juvenile hormone I. Only the 7- and 11-carbons proved to be labeled, fully in accord with prediction. The pathway suggested by these experiments (Scheme II) involved a series of new homologous terpenoid precursors, the central of which was homomevalonic acid. This compound was prepared in isotopic form and shown to be incorporated into the homologous hormones in the predicted fashion.[9]

Experiments with homogenates of corpora allata of *M. sexta* demonstrated the enzymatic formation of 3-hydroxy-3-ethylglutaric acid and homomevalonic acid from acetyl-CoA and propionyl-CoA.[10] Racemic 3-hydroxy-3-ethylglutaryl-CoA labeled with ^{14}C at the 3 position was prepared, and it was shown that only (3*R*)-homomevalonate was produced by the *M. sexta* corpus allatum reductase.[11] While the subsequent homologous intermediates between homomevalonate and juvenile hormones have not been isolated, it is clear that the pathway shown in Scheme II can account for formation of the carbon chains of all the known hormones.

The late stages of hormone synthesis have been investigated with in vitro cultures of corpora allata[12] and with homogenates of these glands.[13] The latter experiments demonstrated the conversion of labeled farnesyl pyrophosphate to hormone (Scheme III). The enzymes from *M. sexta* corpora allata can carry out the methylation of epoxyfarnesenic acid, but not the epoxidation of methyl farnesenate, and so the biosynthetic pathway in this animal follows the right-hand part of the pathway shown in Scheme III. There is indirect evidence that the left-hand branch is followed in locusts

(6) (a) M. Metzler, K. H. Dahm, D. Meyer, and H. Röller, *Z. Naturforsch. B*, **26B**, 1270 (1971); (b) K. J. Judy, D. A. Schooley, L. L. Dunham, M. S. Hall, B. J. Bergot, and J. B. Siddall, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1509 (1973).

(7) (a) D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall, and J. B. Siddall, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2921 (1973); (b) H. Rode-Gowal, S. Abbott, D. Meyer, H. Röller, and K. H. Dahm, *Z. Naturforsch. C*, **30C**, 392 (1975); (c) R. C. Jennings, K. J. Judy, D. A. Schooley, M. S. Hall, and J. B. Siddall, *Life Sci.*, **16**, 1033 (1975).

(8) M. G. Peter and K. H. Dahm, *Helv. Chim. Acta*, **58**, 1037 (1975).

(9) (a) R. C. Jennings, K. J. Judy, and D. A. Schooley, *J. Chem. Soc., Chem. Commun.*, 21 (1975); (b) D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall, and R. C. Jennings in "The Juvenile Hormones", L. I. Gilbert, Ed., Plenum Press, New York, 1976, p 101.

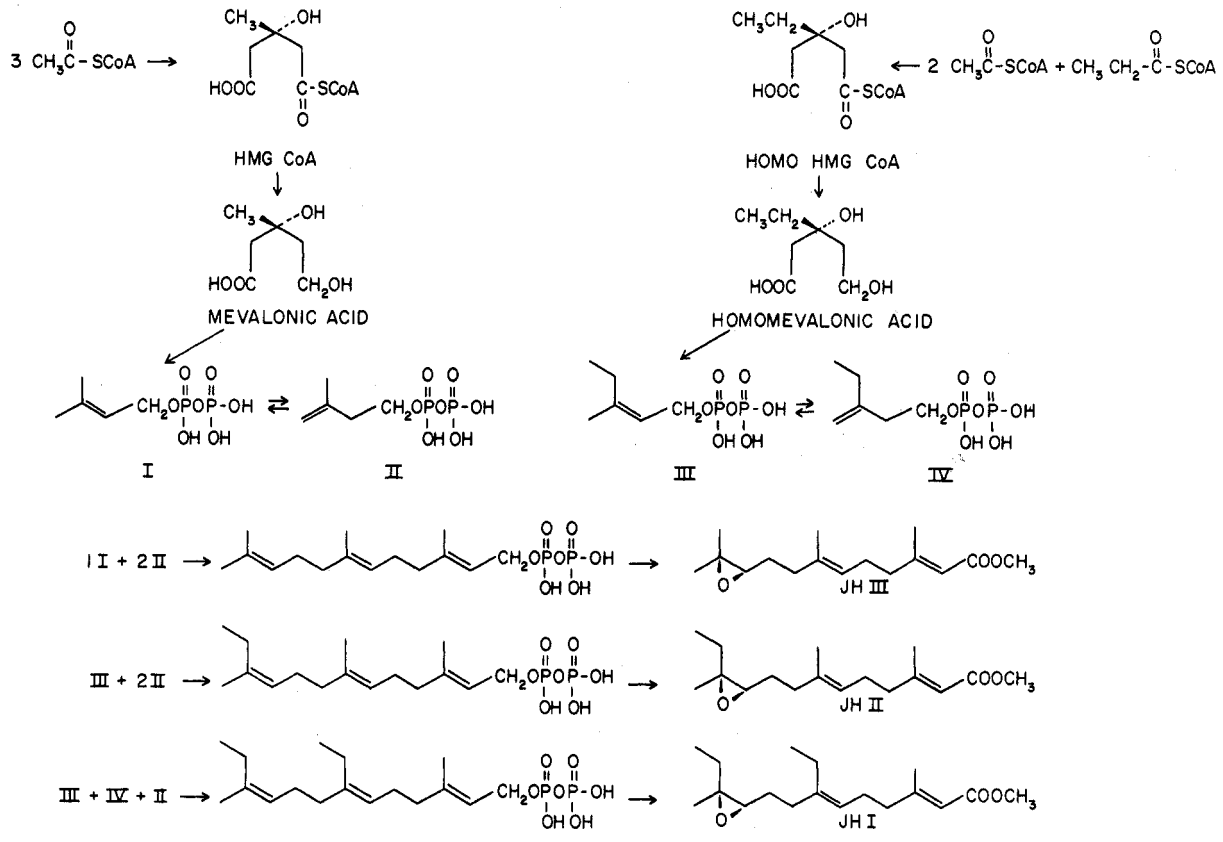
(10) (a) E. Lee, D. A. Schooley, M. S. Hall, and K. J. Judy, *J. Chem. Soc., Chem. Commun.*, 290 (1978); (b) F. C. Baker and D. A. Schooley, *ibid.*, 292 (1978).

(11) B. J. Bergot, F. C. Baker, E. Lee, and D. A. Schooley, *J. Am. Chem. Soc.*, **101**, 7432 (1979).

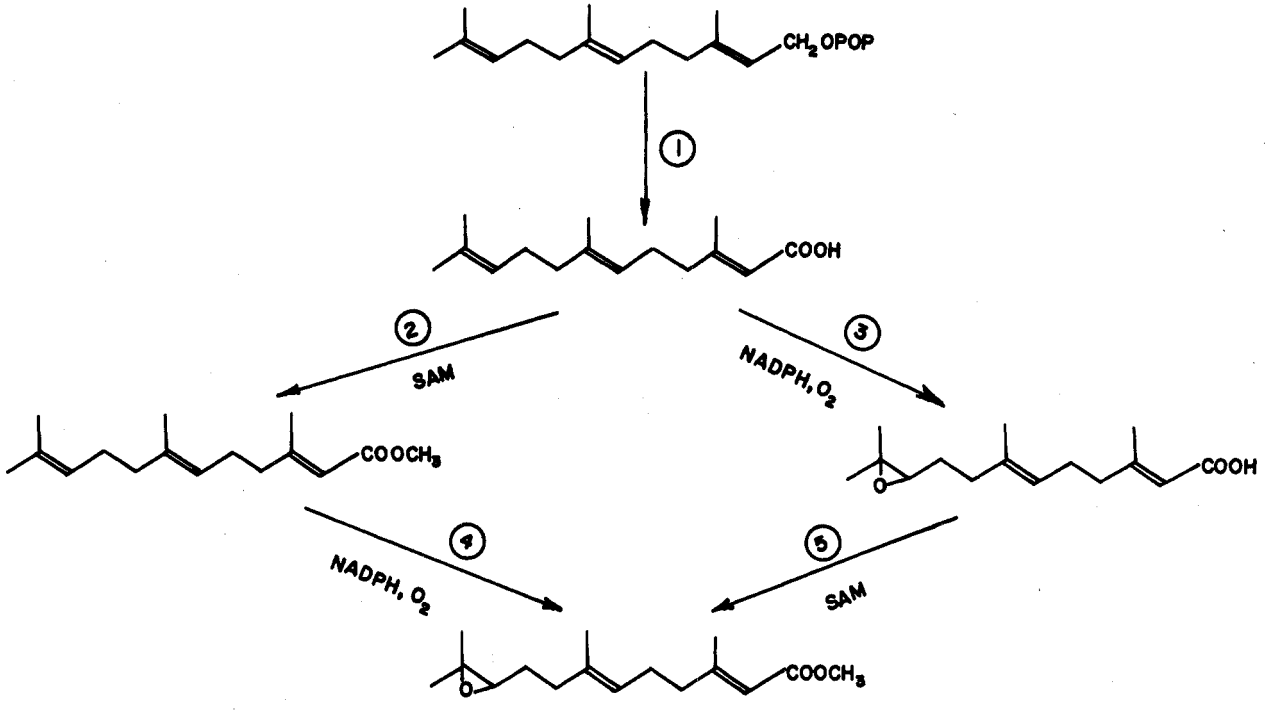
(12) (a) G. E. Pratt and S. S. Tobe, *Life Sci.*, **14**, 575 (1974); (b) S. S. Tobe and G. E. Pratt, *Biochem. J.*, **144**, 107 (1974); (c) S. S. Tobe and G. E. Pratt, *J. Exp. Biol.*, **62**, 611 (1975).

(13) D. Reibstein, J. H. Law, S. B. Bowlus, and J. A. Katzenellenbogen in "The Juvenile Hormones", L. I. Gilbert, Ed., Plenum Press, New York, 1976, p 131.

Scheme II
Postulated Pathway for the Biosynthesis of Juvenile hormones



Scheme III
Late Stages of Juvenile Hormone Biosynthesis



and cockroaches,[12] and homogenates of glands from the cockroach, *Blaberus giganteus*, have been shown to epoxidize methyl farnesenate.[14] It seems likely that different sequences of these final steps will be found in different species, and perhaps both pathways are followed simultaneously in some species.

(14) (a) B. D. Hammock, *Life Sci.*, 17, 323 (1975); (b) B. D. Hammock and S. M. Mumby, *Pestic. Biochem. Physiol.*, 9, 39 (1978).

Control of Hormone Biosynthesis

It is clear that the production of juvenile hormone by the corpora allata is a regulated process and that the rate of production changes during the life of the animal. We know this from classical experiments in which corpora allata from animals at various life stages were transplanted into a susceptible test animal that would respond in a predictable fashion to the presence of the

hormone.[15] Thus, corpora allata from young and mid-stage larvae were generally active, those from pupae generally inactive, and those from adult females again generally active. The activity of the glands in terms of hormone production is often correlated with size of the glands and morphology of the gland cells.[16]

In recent times it has become possible to assess production by removing the glands and incubating them in a tissue culture medium that contains radioactive methionine. The hormone produced is then radioactive and may be easily quantitated.[12] Again, there is often good correlation between hormone production and the physiological stage of the animal from which the glands are taken.[17] It has also been shown that corpora allata do not store hormone; it is released into the surrounding fluid as soon as it is produced.[18]

The functioning of the corpora allata is thought to be influenced in two ways: by nervous signals carried by neurons that connect the glands to the brain and other parts of the neuroendocrine system and by humoral substances carried by the hemolymph.[2,19] In some species cutting the nerves leading to the corpora allata results in a decrease in hormone production while in others it results in an increased or long-sustained output of hormone. This nervous control is presumably mediated by transmitter substances released from nerve endings impinging on corpus allatum cells when an impulse moves down the nerve cell. Membrane receptors activated by the transmitter would then set into action the biochemical response of the cell leading to either increased or decreased hormone production.

Humoral signals might include materials released from cells of the nervous system (neurosecretions) at remote locations and carried to the corpora allata by the hemolymph. There they might interact with membrane receptors or enter the cells to exert either stimulatory or inhibitory effects.[2a] Additional controlling factors might arise in other organs as well. Herein lies a rich field for investigation, probably involving minute amounts of peptides that regulate hormone production. In vitro gland incubation techniques described above should be valuable in identifying and isolating these substances. While we know little about this at present, we have two other types of information about the regulation of hormone production.

The first involves a feedback mechanism by which the animal relates hormone production to the concentration of hormone in the hemolymph (homeostasis).[20] When one corpus allatum is removed from *Leptinotarsa*, the remaining gland increases its production until it is equal to that of the original pair, as judged by hormone production in tissue culture. Furthermore, if the animals are treated topically with hormone, the productive capacity of the glands falls to half or lower. Adult female *Manduca*, on the other hand, maintain the level of hormone production even with massive hormone treatment. It is not at all clear why these two species are so different in this respect, but the answer may lie in the reproductive strategy of each. Compared to *Manduca*, *Leptinotarsa* adults are rather long-lived, and reproduction may be interrupted by diapause, a resting state caused by unfavorable environmental conditions, during which juvenile hormone synthesis is low. Thus, the need to modulate juvenile hormone levels in the adult *Leptinotarsa* may necessitate a feedback control on synthesis.

The second clue we have about regulation of hormone production involves the identification of the rate-limiting process. In *M. sexta*, activity of one enzyme in the synthetic pathway, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, is responsible for the production of mevalonic acid and possibly for homomevalonic acid as well. Activity of this enzyme parallels the production of hormone, as judged by corpora allata gland cultures.[21] We do not know if this enzyme actually determines the rate of hormone production, for until we determine the activity of all subsequent enzymatic steps, we cannot be certain that several enzymes do not become limiting coordinately. However, the synthesis of cholesterol in mammals is thought to be regulated by the activity of HMG-CoA reductase,[22] and it seems likely that the same is true in the case of juvenile hormone biosynthesis. It will be of interest to determine if substances that alter the rate of hormone production do so by affecting the level of HMG-CoA reductase.

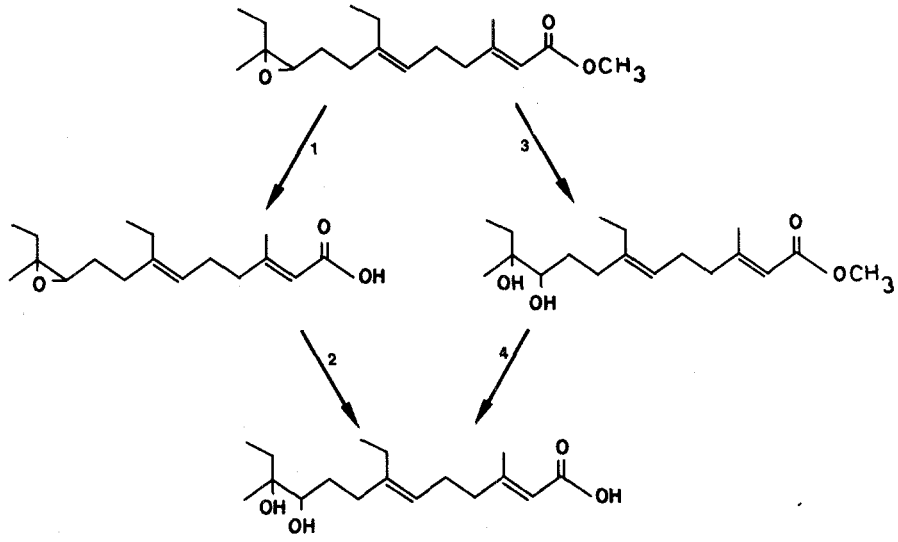
Transport of Juvenile Hormones

Hormone synthesized and secreted by the corpora allata is transported through the hemolymph to the target tissues. Because of the lipophilic nature of the juvenile hormone molecule, it was initially suggested that it might be transported by lipoproteins, in a manner similar to neutral lipids, sterols, and a number of other organic compounds.[23] This suggestion was rationalized on the basis of an assumed water insolubility of the hormone. In fact, the true solubility of these compounds in water had never been accurately determined, and it was shown that juvenile hormones can form aqueous solutions of concentrations up to 5×10^{-5} M.[24] Since the concentration of the hormone circulating in the hemolymph of a larval insect is less than 10^{-7} M, there is actually no a priori reason why the hormone could not be in true solution. Nevertheless, several investigators have demonstrated interactions of the hormone with macromolecules in the insect hemolymph. These carrier proteins can be divided into two classes based upon affinity and binding capacity. Besides low-affinity, low-specificity, and high-capacity interactions with lipoproteins, high-affinity, high-specificity, and low-capacity interactions with specific carrier proteins occur in the insect hemolymph.

Lipoproteins are of major importance in transporting fatty acid esters, e.g., diglycerides, in insect hemolymph. Whitmore and Gilbert[25] first showed that some of the lipoproteins of *Hyalophora gloveri* pupae could bind labeled juvenile hormone and that lipoproteins from adult males contained enough hormone to be detectable

(15) V. B. Wigglesworth, "Principles of Insect Physiology", 7th ed., Chapman and Hall, London, 1972, p 76.
(16) K. Sláma, M. Romaňuk, and F. Šorm, "Insect Hormones and Bioanalogues", Springer-Verlag, New York, 1974, p 27.
(17) (a) S. S. Tobe and B. Stay, *Gen. Comp. Endocrinol.*, **31**, 138 (1977); (b) S. J. Kramer and J. H. Law, *Insect Biochem.*, in press.
(18) S. S. Tobe and G. E. Pratt, *Nature (London)*, **252**, 474 (1974).
(19) L. I. Gilbert, W. Goodman, and W. E. Bollenbacher, *Int. Rev. Biochem.*, **14**, 1 (1977).
(20) H. Schooneveld, S. J. Kramer, H. Privee, and A. van Huis, *J. Insect Physiol.*, **25**, 449 (1979).
(21) S. J. Kramer and J. H. Law, unpublished.
(22) V. W. Rodwell, J. L. Nordstrom, and J. J. Mitschelen, *Adv. Lipid Res.*, **14**, 1 (1976).
(23) L. I. Gilbert and A. Chino, *J. Lipid Res.*, **15**, 439 (1974).
(24) K. J. Kramer, L. L. Sanburg, F. J. Kézdy, and J. H. Law, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 493 (1974).
(25) E. Whitmore and L. I. Gilbert, *J. Insect Physiol.*, **18**, 1153 (1972).

Scheme IV
Pathways for Degradation of Juvenile Hormones in Insects



by bioassay. Several investigators have subsequently made similar observations in a variety of insect species[26] while in other insects it has been demonstrated that hormone is transported by small specific carrier proteins.[24,27] A comparison of the situations in the hemolymph of the tobacco hornworm and the Colorado potato beetle illustrates two extremes.

When the hemolymph of *M. sexta*, treated with an organophosphate to inhibit degradative enzymes, was separated by gel permeation chromatography, the predominant labeled macromolecule had a molecular weight of about 28 000. This carrier protein has been isolated and shown to consist of a single polypeptide chain with a single hormone binding site of high affinity ($K_D = 3 \times 10^{-7}$ M).[28] Studies with a series of hormones and analogues have shown that the binding site is characterized by a sterically defined hydrophobic region with polar sites that recognize the epoxide and ester functions.[29] The binding specificity is restricted to the natural optical isomer of juvenile hormone III.[30] This restricted specificity and high affinity confirm that this molecule is indeed the hormone transport vehicle, and not merely a general lipid carrier.

When the same sort of experiment was carried out with hemolymph from *L. decemlineata*, hormone was associated only with the lipoprotein fraction with molecular weight greater than 200 000. The lipoproteins have a high capacity for binding juvenile hormone as compared with the specific carrier protein of *M. sexta*, but the affinity is low ($K_D \geq 10^{-5}$ M) and lacks specificity for the hormone structure.[31] In *M. sexta* hemolymph, juvenile hormone is associated with lipoproteins only when the specific carrier protein is saturated.[24] Thus, the *M. sexta* system is clearly distinguishable from the nonspecific binding of hormone to lipoproteins found in the Colorado potato beetle and in a few other insect species. In *M. sexta* and other lepidoptera it is likely that specific carrier proteins are of major importance for the control of development. However, in the Colorado potato beetle, which lacks a specific carrier protein, a different regulation system must exist.

The diversity of insect metabolism is illustrated by another example, that of the grasshopper, *Gomphoceras rufus*. Hartmann[32] has shown that juvenile hormone is associated with the lipoproteins in this animal, but that the carrier is a minor component of the lipoprotein fraction and has a high affinity ($K_D = 5 \times 10^{-8}$ M) for the hormone. He has also shown that antibodies raised to this carrier protein, when injected into adult females, interfere with maturation of the reproductive system, thus demonstrating the essential role of the juvenile hormone carrier protein.

Enzymatic Degradation of Juvenile Hormones during Transport

An important aspect of the transport of juvenile hormone is the fact that hemolymph usually contains enzymes that can degrade hormone molecules. The original studies by Slade and Zibitt[33] demonstrating that hormone degradation proceeds via ester hydrolysis and epoxide hydration have been confirmed for several orders of insects. These enzymatic processes are shown in Scheme IV. Some of the reactions may occur in the hemolymph, e.g., reaction 1 in the case of the tobacco hornworm and the Colorado potato beetle, while others occur in the fat body and in other tissues. Since the activity of the degradative enzymes varies widely among different insect species, it is possible that degradative enzyme synthesis or activation may be an important control mechanism for regulation of hormone titers.

The first developmental studies of hemolymph esterases that degrade juvenile hormone in *M. sexta* revealed different activity patterns when juvenile hormone and α-naphthyl acetate were used as substrates.[34]

(26) (a) K. H. Trautmann, *Z. Naturforsch. B*, **27B**, 263 (1972); (b) H. Emmerich and R. Hartmann, *J. Insect Physiol.*, **19**, 1663 (1973); (c) S. D. Bassi, W. Goodman, C. Altenhofen, and L. I. Gilbert, *Insect Biochem.*, **7**, 397 (1977).

(27) (a) S. M. Ferkovich, D. L. Silhacek, and R. R. Rutter, *Insect Biochem.*, **5**, 141 (1975); (b) K. J. Kramer and C. N. Childs, *ibid.*, **7**, 397 (1977).

(28) K. J. Kramer, P. E. Dunn, R. C. Peterson, H. L. Seballos, L. L. Sanburg, and J. H. Law, *J. Biol. Chem.*, **251**, 4979 (1976).

(29) R. C. Peterson, M. J. Reich, P. E. Dunn, J. H. Law, and J. A. Katzenellenbogen, *Biochemistry*, **16**, 2305 (1976).

(30) D. A. Schooley, B. J. Bergot, W. Goodman, and L. I. Gilbert, *Biochem. Biophys. Res. Commun.*, **81**, 743 (1978).

(31) S. J. Kramer and C.A.D. de Kort, *Insect Biochem.*, **8**, 87 (1978).

(32) R. Hartmann, *Roux's Arch. Dev. Biol.*, **184**, 301 (1978).

(33) M. Slade and C. H. Zibitt in "Insect Juvenile Hormones", J. J. Menn and M. Beroza, Eds., Academic Press, New York, 1972, p 155.

(34) G. Weirich, J. Wren, and J. B. Siddall, *Insect Biochem.*, **3**, 397 (1973).

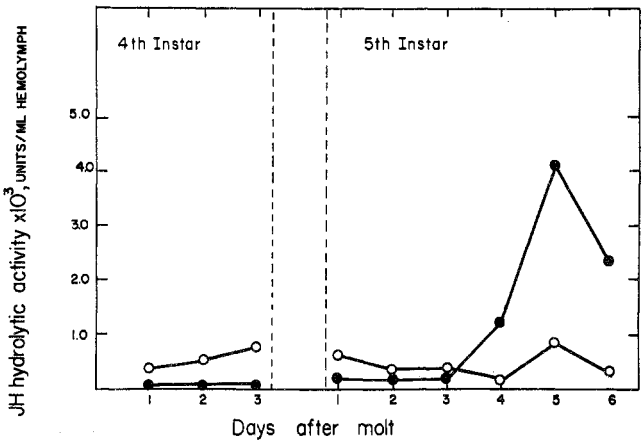


Figure 2. General esterase (open circles) and juvenile hormone specific esterase (filled circles) activities during the last larval instars in the tobacco hornworm (adapted from ref 35).

This indicated the existence of at least two esterase populations with one exhibiting greater specificity for the hormone. Gel permeation chromatography and isoelectric focusing permitted separation of these two esterase populations, one of which possessed high specificity toward juvenile hormone (JH-specific esterases).[35] This group of enzymes was relatively resistant to the organophosphate inhibitor, diisopropyl phosphorofluoridate (DFP), while the other group (general esterases) was inhibited completely. This provided a specific tool for locating and measuring JH-specific hydrolases. When preincubation with DFP was used, the changes in esterase level through the fourth and fifth larval instars were followed. Figure 2 shows that while the level of general esterases fluctuates, that of the hormone-specific esterases remains very low until the fourth day of the fifth instar, when it increases more than 30-fold. It remains at a high level for a short period (1–2 days) and then declines. Similar methodology can be used to determine specific and general esterases in *L. decemlineata*,[36] and Figure 3 illustrates the fluctuation in JH-specific esterase levels at different developmental stages as well as the correlation between esterase levels and hormone titers.

Extensive studies were performed to test the action of esterases on the hormone–carrier protein and hormone–lipoprotein complexes. In *M. sexta* it appears that hormone bound to the carrier protein can be degraded by JH-specific esterases, while general esterases can only degrade free hormone.[35] In addition, developmental studies with *M. sexta* revealed that JH-specific esterases appear at specific stages, suggesting some precise control of synthesis and/or release.[35,37] On the other hand the Colorado potato beetle does not possess specific carrier proteins, and juvenile hormone complexes with high molecular weight lipoproteins are unprotected against hemolymph esterases.[28] Thus, the titer of JH in the Colorado potato beetle is controlled by synthesis and release of hormone by the corpora allata on one hand and enzymatic degradation of hormone by esterases, mainly in the hemolymph, on the other. Determination of the in vitro rate of synthesis

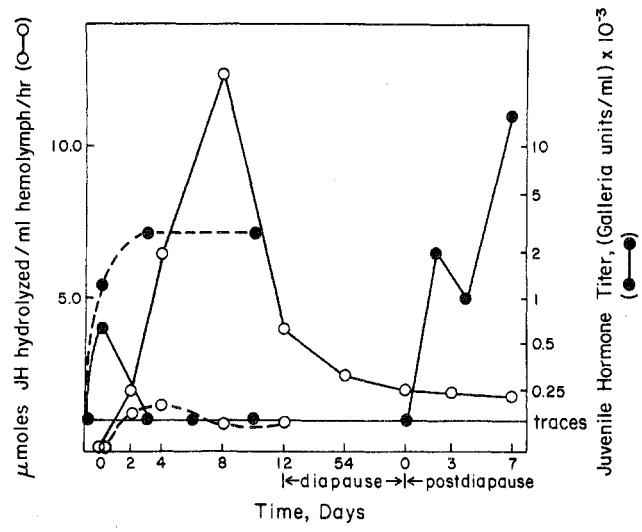


Figure 3. Changes in esterase levels and juvenile hormone levels (determined by bioassay) in the Colorado potato beetle adult maintained under short-day photoperiod (diapause inducing) or long-day photoperiod (from ref 36). Solid lines, short days; dashed lines, long days.

of hormone by corpora allata of the Colorado potato beetle showed a close correlation between CA activity and hormone titer at different developmental stages.[38] Therefore, it can be concluded that synthesis and release from the corpora allata probably primarily govern the titer of juvenile hormone. Only during stages when a low titer of hormone is required, e.g., in beetles reared with a short day to induce diapause, were activities of JH-specific esterase high, thus eliminating all traces of the hormone before the insect enters diapause (Figure 3). For *M. sexta* a somewhat different model is proposed. In this insect it is likely that interactions of JH-specific esterases and carrier proteins, in addition to significant changes in corpus allatum activity, play an important role in regulating JH titers. By suppressing the degradation of hormone by general esterases, which probably exist in all insect hemolymph for some different purpose, the carrier protein allows the animal to function with lower hormone production than in animals that lack specific carriers.

Concluding Remarks

The establishment and maintenance of physiological concentrations of juvenile hormone in insect hemolymph are the result of an interplay between the corpora allata, carrier proteins, degradative enzymes, and those processes that transport hormone into the tissues. While we have learned something of the former processes, we know very little of the mode of hormone entry into cells or what happens to it once it enters. Recent studies indicate that high affinity receptor proteins exist in cell cytoplasm,[39] and probably in nuclei as well,[40] and that binding of the hormone by these proteins is probably essential to the action of the hormone in the cells.

In practical terms, an understanding of all of these aspects of juvenile hormone metabolism may guide future efforts to intervene in the normal regulatory

(35) L. L. Sanburg, K. J. Kramer, F. J. Kézdy, and J. H. Law, *J. Insect Physiol.*, 21, 873 (1975).
(36) C.A.D. de Kort, S. J. Kramer, and M. Wieten in "Comparative Endocrinology", P. J. Gaillard and H. H. Boer, Elsevier, Amsterdam, 1978, p 507.
(37) R. K. Vince and L. I. Gilbert, *Insect Biochem.*, 1, 115 (1977).
(38) S. J. Kramer, *J. Insect Physiol.*, **24**, 461 (1978).
(39) E. S. Chang, T. A. Coudron, M. J. Bruce, B. A. Sage, J. D. O'Connor, and J. H. Law, in preparation.
(40) L. M. Riddiford and T. Mitsui in "Comparative Endocrinology", P. J. Gaillard and H. H. Boer, Elsevier, Amsterdam, 1978, p 519.

functions in the insect. Interference with biosynthesis or its control would result in hormone deficiencies or excesses, either of which might prove detrimental to the animal. The same applies to potential techniques for disrupting transport, degradation, or entry into target cells. Through rational screening several compounds have been identified with such properties. A group of natural compounds (precocene I and II) and chemically related analogues was discovered by Bowers et al.[41] Precocenes appear to destroy juvenile hormone producing cells in corpora allata and thereby cause precocious (hence the name) development of miniature adults in a limited number of insect species. Another compound, ethyl 4-[2-*tert*-butylcarbonyloxy]butoxybenzoate (ETB), described by Staal,[5] is selective as an antagonist of juvenile hormone for a few lepidopteran species (G. B. Staal, personal communication).

Compounds that antagonize juvenile hormones are potentially more useful for insect control than those that mimic them because of less need for critical timing in application, a shorter response time, and the additional inhibitory effects on insect reproduction. The possibility for interference with JH production, transport, and target binding appears to be far from exhausted and may benefit from increased investigation.

(41) W. S. Bowers, T. Ohta, J. S. Cleere, and P. A. Marsella, *Science*, 193, 542 (1976).

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"Ordered" Distribution of Electrically Charged Solutes in Dilute Solutions

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About 40 years ago, Bernal and Fankuchen[1] carried out detailed X-ray analyses of plant viruses (mainly tobacco mosaic virus) in wet and dry gel states and found distinct intermolecular reflections. They also observed similar X-ray patterns for concentrated solutions. They concluded that the virus particles were distributed equidistantly not only in the gel states but also in the concentrated solution. They observed that solutions down to a concentration of 20% showed "as perfect reflection as crystal".

Subsequently, Riley and Oster[2] observed one or more reasonably well-defined small-angle X-ray diffractions at high concentrations of proteins such as bovine serum albumin and suggested that the molecules took up mean positions equidistant from each other.

As a cause of such equidistance in solutions, Bernal and Fankuchen suggested *attractive* forces between the particles balancing *repulsive* forces (probably of electrostatic nature) and pointed out a similarity to the interaction of metal atoms with their positive nuclei and clouds of negative electrons.

Generally speaking, the solute distribution in solution is the direct outcome of solute–solute, solute–solvent, and solvent–solvent interactions. The complete description of the distribution is sine qua non for thorough understanding of solution properties. Obviously, potential calculations leading eventually to prediction of physicochemical properties can be possible only when proper assumptions of solute distribution are made. One example is the theory of strong electrolyte solutions by Debye and Hückel,[3] who assumed the Boltzmann distribution for ions and obtained satisfactory agreement with experiments at high dilutions. Polyelectrolyte dilute solutions have often been approached in terms of the so-called cell model for macroion distribution,[4] although agreement with experiments has been much less satisfactory for various reasons. Liquid crystals constitute another well-known case which demonstrates the importance of correlation between solute distribution and solution properties.

It would readily be accepted that solute species, electrically charged or not, are distributed regularly in highly concentrated solutions. This regular spacing might be expected to be destroyed by thermal motion progressively as the solute concentration is lowered. Recent studies show, rather unexpectedly, that some degree of ordering still persists even at relatively low concentrations of electrically charged species such as simple ions, macroions, proteins, ionic micelles, and polymer latex particles. Although many unsolved problems await future investigation, especially for macroion systems, we wish to examine here relevant experimental work on various solution systems in order to provide a basis for a (not fragmentary but) unified interpretation on the structure of dilute solutions of charged solutes.

Thermodynamic Measurements

The thermodynamic properties of simple electrolyte solutions such as the mean activity coefficient, osmotic

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(1) Bernal, J. D.; Fankuchen, I. *J. Gen. Physiol.* **1941**, *25*, 111.
(2) Riley, D. P.; Oster, G. *Discuss. Faraday Soc.* **1951**, *11*, 107.
(3) Debye, P.; Hückel, E. *Phys. Z.* **1923**, *24*, 185.
(4) Oosawa, F. "Polyelectrolytes"; Marcel Dekker: New York, 1971.

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