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A MINIMAL MODEL OF METAMORPHOSIS: FAT BODY COMPETENCE TO RESPOND TO JUVENILE HORMONE

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ARGUMENTS FOR A MINIMAL MODEL

The ontogeny of larval forms of invertebrates and vertebrates has fascinated embryologists and evolutionary biologists for centuries (Gould, 1977). We are now at a stage when appropriate choices of models of metazoan developmental phenomena may tallow us to understand them on a mechanistic and molecular level. Success in this venture may depend on the ultimate complexity of the model chosen to study and for this reason potential models of minimal complexity should be sought.

The scientific literature is replete with examples in which concentrating on a simpler system affords rapid progress in a subject. *Neurospora* and later *E. coli* revolutionized the study of genetics and biochemistry. The further search by geneticists for simpler systems led to the use of viruses and ultimately to the simplest of all replicating organisms, the RNA-coliphage. For some strains of this phage, which has only 3 genes, science is in the enviable position in which it can claim an almost total inventory of an organism's molecules and behavior.

While microbial models for metamorphosis do exist, i.e., bacterial sporulation (Losick, 1973), or cellular slime mold morpho-genesis (Bonner, 1967), one must eventually ask the general question of whether slime mold, amphibian and insect metamorphosis are based on the same mechanism. For this reason it is useful to explore minimal models of metamorphosis at a number of levels of complexity in a variety of organisms.

The phenomenon of metamorphosis connotes a change of form associated with a change in life style. In cellular slime molds the change is from a unicellular to a multicellular form, brought on by starvation. In amphibians the change usually accompanies the transition from water to land and is most dramatically represented by the metamorphosis of tadpole to frog, which involves both morphological and physiological adaptations to a new ecology· Changes in feeding, excretory physiology and respiratory physiology are among

the dramatic shifts. Sexual maturation, the transition to a re-productively competent life style, is not accompanied by radical morphological changes in vertebrates. Changes which do occur at puberty in the higher vertebrates, birds and mammals, usually involve development of secondary sexual characters such as mammary glands or changes in plumage, developmental changes which do not have the dramatic qualities which have been labeled metamorphosis. Interestingly the evolution of metamorphosis in both insects and amphibians may have occurred at the same time, in the Devonian era, 350-400 million years ago, as a response to the severely fluctuating ecological conditions (Wigglesworth et al., 1963; Wigglesworth,1976).

Among insects the phenomenon of metamorphosis is associated with maturation from a larval to adult form concomitant with a cessation of further molting, and is restricted to the higher, winged, species. The evolution of the metamorphic process is linked to the origins of flight but is shrouded in obscurity due to a poor fossil record (Wooten, 1976). The adults of the earliest known winged insects, from the Carboniferous period, already possessed two pairs of perfect wings (Snodgrass, 1952). At that stage of evolution the wings were the only exoskeletal structures other than genitalia which differentiated adults from larvae (Clarke, 1973). Since, at that time, there already existed two radically different ways of articulating the wing to the body, it is entirely possible that there was more than one independent origin of flight and, likewise, of metamorphosis. However, arguments to the contrary abound (Wigglesworth et al., 1963).

Due to the confusion of the fossil history, if a unified theory of insect metamorphosis is to be established, we must deal with the comparative morphology, physiology, and development of extant forms. The exact correspondence of developmental stages in ametabolous, hemimetabolous and holometabolous types of insect development is as yet unresolved (Hinton and Mackerras, 1970).

Indeed the resolution of the correspondence of metamorphosis and stages in the different groups may come from more detailed knowledge of the regulation of the mechanism(s) of metamorphosis in higher insects and how these mechanisms relate to the primitive regulatory system found in the closest relatives of the Pterygotes among the Apterygotes, the Thysanura (Snodgrass, 1952).

In apterygote insects little or no external metamorphic changes are noticeable in the larval to adult transition. The basic ecology and physiology of the maturing animal does not change, and if an observer did not notice the reproductive behavior and egg production, it would be hard to tell that an important transition had occurred. Silverfish (Thysanura) development is a typical example of this "ametabolous" type of maturation, Fig. la. At the transition from last larval to first adult stage some changes in scale pattern do occur, and these small differences may have been amplified by natural selection into the types of differences which preadapted a Thysanuraform ancestor to give rise to a metamorphic, winged line of descent (Wigglesworth et al., 1963).

In modern day Thysanurans the pattern of molting cycles of the larval phase is continued in the adult, but each female adult molting cycle is preceded by a yolk deposition and ovulation cycle (Watson, 1964). Ovulation and molting alternate in a mutually exclusive pattern for the remainder of the life of the female sex of this ametabolous insect. This alternating pattern in the adult is under neuroendocrine control and could serve as a model of a primitive control system from which all the higher insect molting and reproductive control systems are derived.

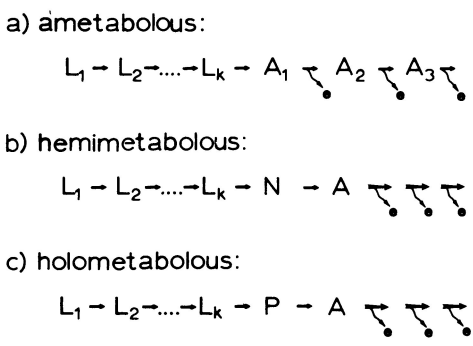

Fig.1 Three major types of insect development: a) Ametabolous development typified by the silverfish, b) Hemimetabolous development typified by the cockroach; in some hemimetabola such as termites and grasshoppers there may be more than one stage with wing pads that could be called nymphal; c) Holometabolous development typified by flies, moths and butterflies, and beetles Abbreviations: '**A**' adult stage; '**L**' larval stage; '**N**' nymphal stage (=last instar larva); '**P**' pupal stage; '**e**' eggs.

At the other extreme the holometabolous insects undergo dramatic changes in ecology, physiology and morphology at the larval to adult transition, Fig. 1c. Their so-called "complete" metamorphosis is so extreme that an intermediate stage, the pupa, is required to accommodate the replacement of larval structures by adult structures (Hinton, 1963). In the Diptera the changes from maggot to fly are such that few functional larval tissues survive in the adult. The fly tissues are derived from nests of cells, imaginal discs, which do not participate in forming larval structures (Poodry, 1979). In these extreme cases it is inappropriate to talk about a particular tissue in terms of its larval to adult transition. Metamorphosis is accomplished by the programmed death of one set of tissues, the larval set, and growth and terminal differentiation of a second set, that of the adult. In those holometabolous groups in which a tissue does function continuously in larva, pupa and adult, such as in the lepidopteran abdominal epidermis (Willis, 1969 and this volume), the external morphology, ultrastructure and biochemistry of the tissue undergo numerous changes. As adults the majority of holometabolous insects still produce eggs in batches in a cyclical fashion; most with the involvement of juvenile hormone, JH, and some as suggested by various investigators, with the involvement of both JH and ecdysone (for reviews see Hagedorn and Kunkel, 1979; Engelmann, 1979).

These holometabolous metamorphic systems, though they serve well to demonstrate and elucidate the neuroendocrine controls and phenomenology of metamorphosis, are too complex to allow an understanding of the mechanism of metamorphosis at the biochemical level. The basic problem as I see it is to separate those biochemical processes which are part of the larval, pupal or adult phenotypes from those which are important for the transition between these stages. In holometabolous development too many of the observed phenomena are likely to be attributes of a stage phenotype rather than a part of the ontogenetic mechanism of transition. Since the larval to pupal and pupal to adult molting cycles are one of a kind, i.e., occurring only once in the normal development of an individual, it is additionally hard to distinguish the stage specific molting physiology from the metamorphic process.

On the other hand the hemimetabolous type of metamorphosis, Fig. lb, as exemplified by the cockroach, is, in many respects, a minimal model of metamorphosis. The ecology of the larva and adult is often quite similar, and most of the functional larval tissues continue to be functional in the adult. The metamorphosis

is often described as 'incomplete' or 'gradual'. A transition stage between immature and adult, the nymph, is distinguished by some investigators in specific groups of hemimetabolous insects. Although certain schools eschew the use of the term 'nymph' I find it useful. The nymph, as opposed to a larva in general, has developed wing pads and in some groups, including the cockroaches, is diagnostic of the fact that metamorphosis will occur with the next molt. I will subsequently refer to the last instar larva as a nymph, while retaining the more traditional term 'penultimate larva' for the next to last larval instar. The nymph stage has been given special meaning and formalized with relation to the caste system in the termites, immunologically close relatives of the cockroach (Kunkel and Lawler, 1974). The termite nymph has already undergone some metamorphic changes and is restricted in its developmental potencies; it is capable of becoming a functional reproductive but not a soldier (Wilson, 1975).

Since the majority of tissues in the hemimetabola are continuously functional through the larval, nymphal and adult stages, one can ask in real terms how particular tissues are changed in function through metamorphosis. If the hemimetabolous and holometabolous mechanisms of metamorphosis are basically the same, then by choosing the simpler hemimetabolous model we have enriched for the details of the metamorphic mechanism rather than the details of the changed stages.

Development vs. Physiology

Even after establishing a hemimetabolous minimal model of metamorphosis, there remains the problem of separating the important events of metamorphosis from the physiology of the molting process. Growth and development of arthropods are inextricably tied to the molting process, during which the old cuticle is replaced by a fresh, usually larger, and, if metamorphosis is occurring, geometrically different exoskeleton. The problem of deciding what is molting and what is metamorphosis is most clearly explained by discussing the epidermis. Even during isometric growth, which is predominant during larval-larval molt cycles, Fig. 2a, the cockroach epidermis undergoes distinct differentiative and proliferative phases of mitosis (Kunkel, 1975a). The differentiation of new sensory and glandular organules in the expanding epidermis occurs during the intermolt phase of the stadium. Proliferation of the generalized epidermal cells, contributing to the expansion, occurs at the beginning of the molting phase of the stadium but prior to apolysis. In the later part of the molting phase, after apolysis, the epidermal

cells go through their program of digestion of old cuticle and deposition of new cuticle.

The intermolt phase during which the determinative cell divisions for new structures occur, can be expanded by various developmental processes including regeneration, Fig. 2b (Kunkel, 1975a; 1977), and metamorphosis, Fig. 2c. The process of metamorphosis involves changes in all phases of the stadium. However, in the cockroach the length of the molting phase remains relatively constant despite any type of lengthening of the entire stadium, seemingly due to a stereotyped program of epidermal molting behavior initiated in an all-or-none fashion by the brain-prothoracic gland endocrine axis.

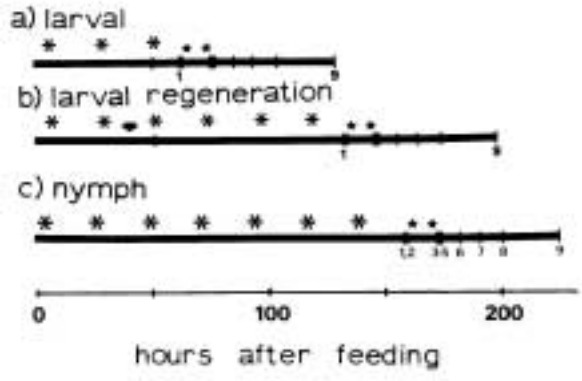

Fig.2 Timing of events during molt-intermolt cycles of the German cockroach, *Blattella germanica*. Periods of proliferative (stars) and differentiative (asterisks) cell divisions are illustrated for a) premetamorphic IV instar larvae, b) IV instar larvae regenerating a leg autotomized at 40 hours after feeding (arrow), c) metamorphosing nymphs. Key to the molting cycle events: 1) brain critical period, 2) regeneration critical period, 3) prothoracic gland critical period, 4) apolysis, 5) end of epidermal mitosis, 6) bristle growth initiation, 7) bristle growth termination, 8) muscle attachment release and 9) ecdysis.

Thus, the allometric changes that occur on a biochemical and morphological level during metamorphosis must be viewed against the backdrop of the cyclical molting physiology (Kunkel, 1975a; 1975b; Duhamel and Kunkel, 1978) that dominates insect growth and development.

The Fat Body as a Metamorphosing Tissue

The epidermis, responsible for the synthesis and secretion of the exoskeleton, has received most attention in studies of metamorphosis. Unfortunately, metamorphosis of this tissue is usually evaluated by its secretion of a cuticle at the end of the molting phase, and local changes in shape and coloration of this structure are difficult to quantify. The fat body, another system which undergoes metamorphic changes may be better tissue in which to study this phenomenon. The fat body is the source of the majority of serum proteins (Wyatt and Pan, 1978) including the JH

binding protein (Nowock et al., 1975), storage proteins, such as calliphorin (Thomson, 1975) and cockroach larval specific protein (Kunkel and Lawler, 1974), and the vitellogenins (Pan et al., 1969). The secretion of these proteins by the fat body constitutes a phenotype which can be continuously monitored in its transition from a larval to an adult pattern. The metamorphosis of the fat body in the holometabola, like epidermal metamorphosis, is dramatic, involving changes in tissue identity, morphology and physiology, which make comparisons of larval and adult fat bodies difficult to interpret.

On the other hand, the fat body of the cockroaches and other hemimetabolous groups remains morphologically intact throughout metamorphosis. While the epidermis is structurally complex and its metamorphosis involves a variety of localized changes, the fat body is made up of only a few cell types and has the potential of reacting in a uniform manner to the forces of metamorphic change: a bio-chemically tractable problem.

The Fat Body as a Minimal Tissue Model of Metamorphosis

It is convenient to assess a major aspect of the in vivo synthetic activity of the fat body by monitoring the concentration of serum proteins in the hemolymph. All of the major serum proteins of the cockroach are large, with molecular diameters greater than 100 Angstroms (Kunkel and Pan, 1976). In the majority of the larval and adult stages there is very little turnover of this group of proteins. As a result, it is possible to follow the increases in concentration and labeling of the major serum proteins seen during each molting cycle (Duhamel and Kunkel, 1978; Duhamel, 1977) and interpret them as accumulations of newly synthesized and secreted protein.

About 24 hours prior to each ecdysis in *B. germanica,* all of the accumulated large serum proteins are cleared from the circulation in a precipitous fashion. Even injected foreign proteins such as horse ferritin and *E. coli* beta-galactosidase, which do not turn over at an appreciable rate during the first three quarters of each stadium, disappear rapidly as ecdysis approaches (Duhamel, 1977; Duhamel and Kunkel, in preparation). In addition, the protein storage granules of the fat body also disappear as ecdysis approaches, and do not reappear until about 48 hours after feeding in the next stadium (Kunkel, 1975a). At present, the best guess as to where these serum and fat body protein resources are going at this time is into the production of the new cuticle. The analogous (and possibly homologous) storage proteins in the holometabola (Thomson, 1975; Wyatt and Pan, 1978) contribute to the general tissue remodeling which

occurs at metamorphosis.

Upon this background of cyclical synthesis of serum proteins by the cockroach fat body there occurs a metamorphosis of the capacity to synthesize and secrete the larval serum protein, LSP, and vitellogenin, Vg. The ability of the fat body to secrete LSP into the serum disappears at the metamorphic molt (Kunkel and Lawler, 1974), while Vg appears for the first time shortly after the first feeding following metamorphosis. This reciprocal change in secretion of two major serum proteins represents the major change of secretory behavior expressed by the cockroach fat body due to metamorphosis. If the process by which LSP secretion is turned off and Vg secretion turned on could be elucidated, we might have a better understanding of metamorphosis in general. Since we know that the synthesis and secretion of Vg in the cockroach is under the control of juvenile hormone (Engelmann and Friedel, 1974), it is also appropriate to ask when and how the fat body becomes competent to respond to this hormone.

The fat body of hemimetabolous insects is an ideal minimal tissue model of metamorphosis. A major larval gene is turned off and a major adult gene turned on while the gross morphological structure is retained intact.

ESTABLISHMENT OF THE COCKROACH MODEL

Heterogeneous Metamorphic Rates of the Cockroach

Despite the fact that feeding can be used to synchronize each molting cycle of a number of species of cockroach (Kunkel, 1966; 1977), the development from egg to adult is not entirely predictable. Cockroaches have been observed to undergo a variable number of molting cycles before reaching the adult stage. The number of instars in the cockroach life cycle has been shown to be affected by various physiological stresses (e.g., antennal amputation (Pohley, 1962), leg amputation (O'Farrell and Stock, 1956)) and ecological factors (e.g., nutrition and colony density (Wharton et al. , 1968)), as well as being different in each sex (Kunkel, unpublished). In cultures in which these extrinsic variables have been minimized or controlled (Kunkel, in preparation), there still remains a substantial variability in the instar of metamorphosis. Genetic selection canalizing for a particular instar of metamorphosis under a particular set of conditions has provided strains in which larvae can be counted on, with high reliability, to become adults at a particular instar, Fig. 3. An example of the progression of such a genetic

selection program is given in Fig. 4. Some strains are rapidly canalized to metamorphose at the particular instar selected for; some strains are more reluctant to cooperate and persist in spreading out the instar of metamorphosis among two or three consecutive instars.

It seems clear that the instar of metamorphosis is a polygenic trait in the cockroach with a good deal of environmental input involved in its expression. Even in highly inbred lines the proportion of individuals metamorphosing at a particular instar, though relatively constant for one set of conditions, can be varied by changing parameters such as the size of the culture container, the amount of food and water available, or the temperature. Clearly our ability to control the instar of metamorphosis by manipulating culture conditions can be a valuable asset in elucidating the neurohormonal controls of the metamorphic process (cf. Nijhout and Williams, 1974). Using uniform, optimum culture conditions combined with canalized strains with reproducible metamorphic patterns, it is now possible to approach the study of the metamorphic process itself. Our ability to assume a future schedule of development for an individual animal is crucial to this study of the early phases of metamorphosis.

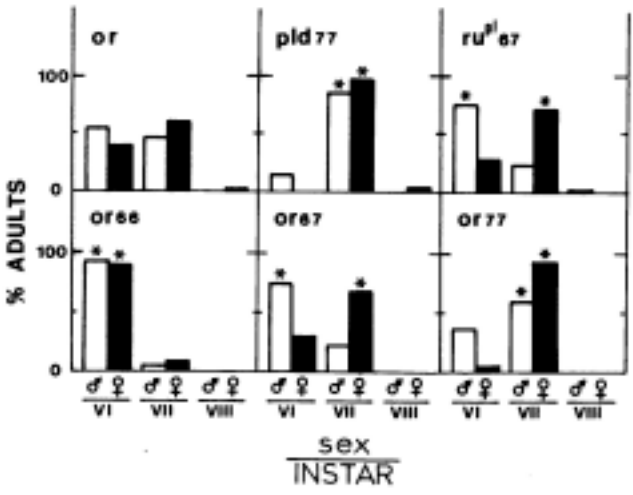

Fig. 3 Instar canalized strains of *Blattella germanica* compared to an unselected strain, *ro*. The instars genetically selected for in each strain are indicated by asterisks. The three strains derived from or illustrate the results of eight generations of selection for the indicated adults and illustrate in strain *or*67 the potential of sexual dimorphism in instar number that is not apparent in the parent strain.

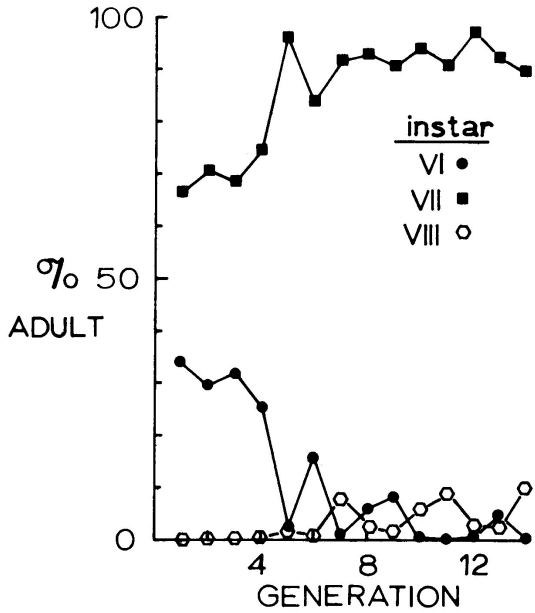

Fig.4 Canalization for seventh instar adults of *Blattella germanica.* The original strain was offspring of a cross between the *or* bearing strain and a New York strain (Kunkel, 1966). At each generation only the VII instar adults were allowed to mate and contribute to the next generation.

Normal Pattern of Vitellogenesis in *Blattella germanica*

Vitellogenesis in adult *B. germanica* is under the extrinsic control of feeding (Kunkel, 1966; 1973) and the intrinsic control of JH (Kunkel, 1973; Kunkel and Pan, 1977). Vg appears in the serum of normal adult females between 12 and 24 hours after feeding commences, Fig. 5, reaches a peak concentration in the serum by 48 hours, and starts declining after ovulation during day 6. Vitellin deposition in the terminal oocytes starts in earnest about 36 hours after feeding, and during the next 5 days the oocytes continue to grow, removing the Vg from the serum by specific adsorptive pinocytosis (Anderson, 1970; Kunkel and Pan, 1976). In order to observe the true Vg secretory capability of the fat body, one may examine secretion in ovariectomized females, Fig. 6, in which the Vg accumulates in the serum and is, initially, only slowly degraded. The Vg in these animals

approaches 8% of the serum by weight and after the time at which they would normally ovulate there is a decrease in concentration. However, their blood volumes are increasing in such an unpredictable fashion at this time that it is difficult to interpret changes in the Vg titer. Vg is synthesized indefinitely in these ovariectomized animals, as has been described previously in other species (Engelmann, 1978). Additional pathology due to the ovariectomized condition has been discovered in the corpora allata (Scharrer, this volume).

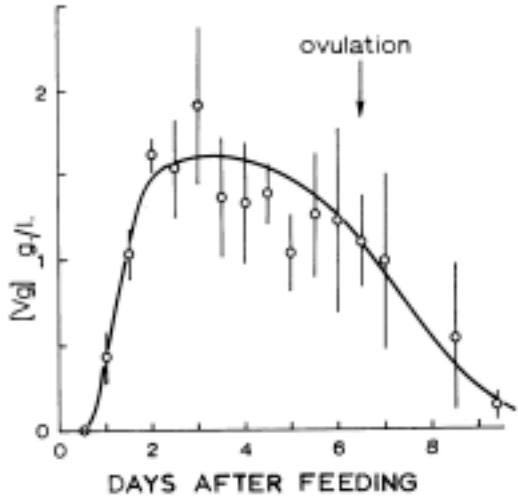

Fig.6 Ovariectomized *Blattella germanica* serum vitellogenin (Vg) titer. Animals were ovariectomized in the nymphal stage, allowed to metamorphose to the adult, starved for one week after the metamorphic ecdysis then fed at 30° C. Vg was measured as in Fig. 5. The mean and range of five observations (= approximate 95% confidence interval of the mean) are plotted. The titer for normal females is included for comparison.

Metamorphosis of Vitellogenic Competence

The vitellogenic response of the adult animal to JH is interesting in a biochemical and physiological sense as an example of hormonal induction of a specific macromolecule. Recent reviews cover this rapidly developing area (Engelmann, 1979; Hagedorn and Kunkel, 1979). However, the more interesting, but rarely examined developmental phenomenon is how the larva, which initially does not produce Vg in response to JH, metamorphoses to the vitellogenically competent state. This can be timed by injecting immature cockroaches with JH at different stages and

asking when they become capable of producing Vg (Fig. 7). The nymph (i.e., last instar larva), early in its stadium accumulates less Vg in its serum within 24 hours after JH injection than does the late nymph. Late penultimate instar larvae (actually pharate nymphs) secrete even less Vg at equivalent JH doses and earlier stages secrete none. These results may be taken to suggest a gradual development of competence to respond to JH during the nymphal stage (Kunkel and Pan, 1977), but a closer look at the kinetics of the vitellogenic response gives a slightly more complex picture (Fig. 8). When the secretion of Vg into serum is followed at finer time intervals during the nymphal instar it is seen that there is a 16 hour lag in Vg appearance after JH injection which is independent of the age of the injected nymph.

This nymphal lag is almost three times longer than the six hour lag in Vg secretion seen when adult females carrying an ootheca are injected with JH.

This difference in lag time may be a reflection of the difference between a primary and secondary response to a hormone, as has been described for the estrogen induction of ovalbumin in chicken oviduct (Schimke et al., 1973). However, attempts to decrease the lag in Vg secretion in response to a JH injection late in the nymphal instar by application of submaximal doses early in the instar were unsuccessful. Irrespective of the meaning of the latter result, the action involved in establishing the difference in lag time between nymph and adult may represent a significant portion of the metamorphosis of the fat body. The short time span involved in the change of a primary to a secondary response, and the lack of substantial gross morphological changes in the fat body during the primary response, may make this fat body phenomenon an important model system for the examination of the development of a hormonal response.

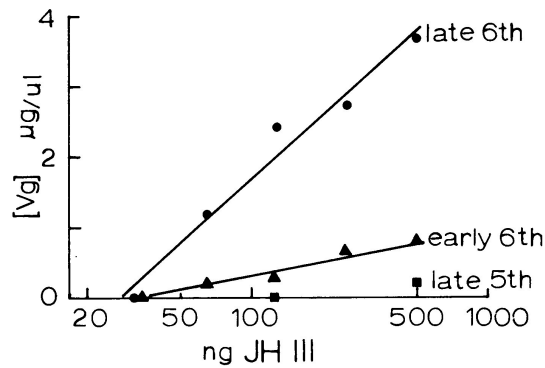

Fig. 7 Juvenile Hormone (JH) induced serum vitellogenin (Vg) titers in larval female *Blattella germanica.* JH III was injected in various doses into early and late VI instar nymphs and late V instar larvae (= pharate nymphs). Vg was measured as in Fig. 5. Each point represents the mean for eight animals.

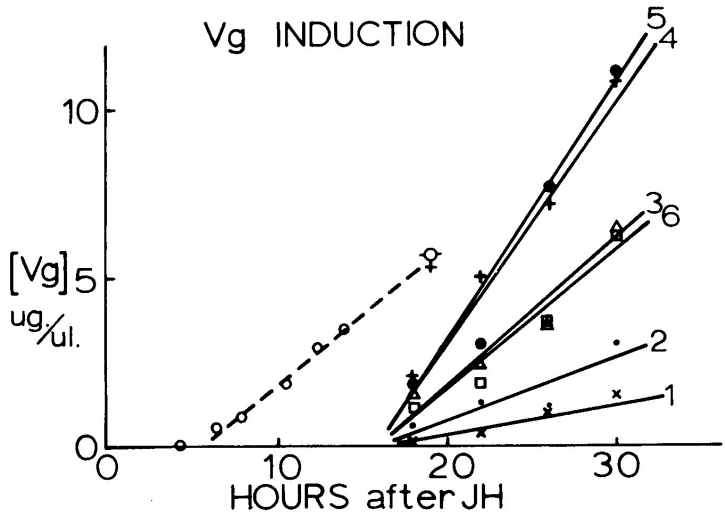

Fig.8 Kinetics of serum vitellogenin (Vg) titer increase in response to juvenile hormone injection. One microgram of JH III was injected into *Blattella germanica* nymphs on days one through six after feeding (solid lines labeled with day of injection) and into adult females carrying oothecae eleven days post ovulation (dashed line). Each point represents the mean of six animals. Analysis of variance of the nymphal data suggests that there are no significant non-linear components to the Vg accumulation data over the time span observed and that each curve extrapolates back to 16 hours after JH injection. The corresponding lag in Vg appearance for the adult females is approximately 6 hours.

Intrusion of Molting Physiology on Metamorphosis

The induced rate of secretion of Vg into the serum increases steadily during the nymphal instar until day 5 (Fig. 9), after which the response to injected JH declines. The early increase in response parallels the increasing capacity of the fat body to secrete all the serum proteins (Duhamel and Kunkel, 1978). At first the decline in JH sensitivity after day 5 was disconcerting because it appeared to be a reversal in the metamorphic process. However, on further analysis, it proved to be another of the properties of the fat body which is sensitive to the stage of the molting process. Day five in these animals is the day when the molting cycle is initiated by the joint action of the brain and the prothoracic glands (Kunkel, 1975a). It is also the time at which the fat body is responding to the molting hormones by an accelerated increase in secretion of serum proteins (Kunkel, 1975b; Duhamel and Kunkel, 1978). The physiology of the fat body during this later phase of the stadium is dominated by the

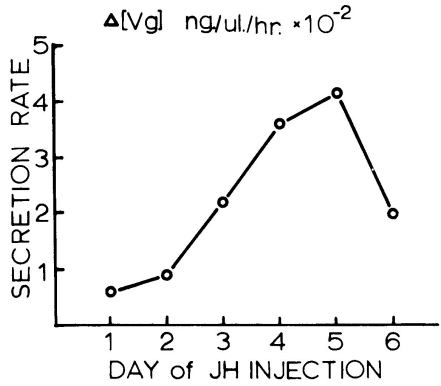

Fig.9 Secretion rates of vitellogenin (Vg) into the serum of larvae injected with JH on different days of their nymphal stadium. The slopes of the lines in Fig. 8 are plotted against the day of JH injection.

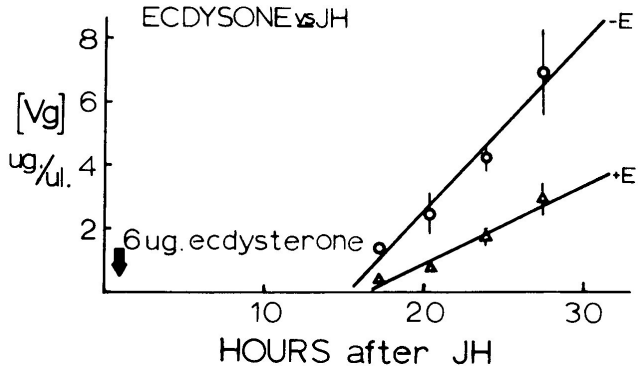

Fig.10 Ecdysterone inhibition of JH induced secretion of vitello-genin (Vg) in VI ins tar larvae of *Blattella germanica.* A single dose of 6 µg ecdysterone was injected one hour after JH injection on day four of the nymphal instar. Vg was measured as in Fig. 5. Each point represents the mean (+ standard error of the mean) for five animals.

molting process. To test directly whether the molting hormone, ecdysone, has an inhibitory effect on JH induction of Vg secretion, nymphs which had been feeding for four days were injected with JH and then injected one hour later with ecdysterone (Fig. 10). The ecdysterone injection, which contained sufficient hormone to induce a normal molting cycle (Kunkel, 1975b; 1977), clearly inhibited the JH response by 50%. In further studies on another species, *Periplaneta americana*, it has

been possible to totally extinguish the JH vitellogenic response in nymphs with ecdysterone (Storella and Kunkel, in preparation).

JH Antagonism of the Molting process

The antagonistic effect of ecdysteroids on the JH response, which has been observed in both hemi- and holometabolous orders of insects (see review by Engelmann, 1979), is paralleled in the cockroach by an antagonism of JH to the molting process. Figure 11 illustrates the response of nymphs to injections of 1 µg of JH-I on days 1 through 9 after last instar feeding begins. The animals pictured are the adults which resulted from molting under the influence of JH. Each column of animals in the figure represents an injection-day group and the rows illustrate the relative timing of ecdysis. Day 1 and day 5 through 9 injected animals did not delay molting at all, but day 2 through 4 injected groups were progressively delayed in their ecdysis times. Also, day 1 through day 4 animals were minimally and unevenly juvenilized by the JH injection, while day 5 through 9 animals were juvenilized in a *status quo* sense. For example, in day 5 animals, adult features such as male tergal glands which undergo most of their formative mitoses during the expanded metamorphic intermolt phase (Fig. 2c), appear normal: their morphological status is determined prior to JH injection. However, features such as the wings, which undergo extensive mitoses at the beginning of the molting phase, are severely stunted by the juvenilizing effects of a JH injection at a time when their mitoses are scheduled to occur. By day 7 the wings and all other structures appear to have been determined since JH injections at this time or later have no gross juvenilizing effects save for cuticle coloration. Despite the adult character of the exoskeletal structures which the 7 day nymphal epidermal cells are poised to secrete, JH injected at this stage is able to induce the cells to lay down a larval melanic pattern of coloration in all the secreted structures. This stepwise metamorphosis of the epidermal cells, which can be visualized by the effect of JH injections at various times during the nymphal instar, is encouraging in that it may also be possible to see such a series of stages in the developing fat body.

Although the groups which delay their molting cycles do not show a uniform juvenilization, the animals of groups 5 through 9 do respond consistently. The ability of JH to uniformly affect the epidermis may extend to internal tissues such as the fat body. For instance, all of 15 nymphs injected with 0.5 µg of JH on day 5 after feeding were juvenilized to the same extent (Fig. 12). When they were fed as adults they produced no eggs, and, on

dissection two weeks later, their ovaries showed no signs of yolk deposition and no Vg in the serum. The JH injection had produced a uniform batch of sterile females. It is not known where the physiological lesions are which prevent a normal reproductive cycle in these particular animals, but it is obvious that this type of preparation may be a valuable aid in our experimental dissection of the metamorphic process. Indeed, this kind of stepwise control in a morphologically unchanging structure such as the fat body, which is responsible for Vg production may permit us to better understand metamorphosis at the molecular level.

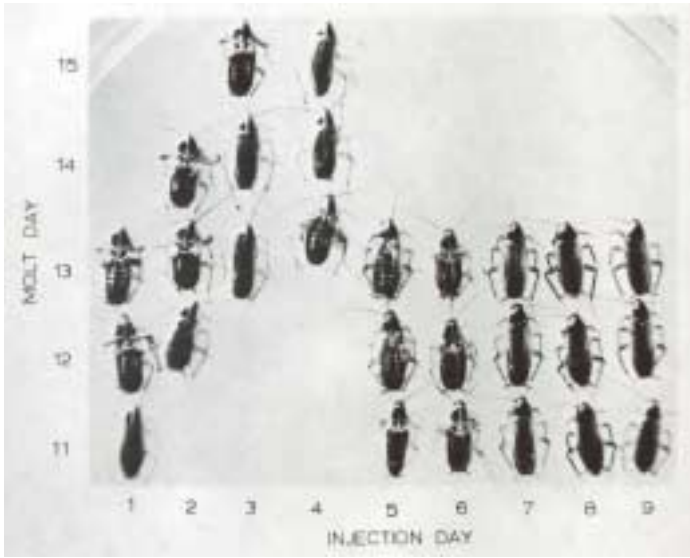

Fig.ll Molting and juvenilization record for groups of fifteen *Blattella germanica* nymphs injected with 1 µg JH-I on days one through nine (columns 1-9) of their metamorphic stadium. The rows labeled 11 through 15 are the days after feeding during which the animals in that row underwent their metamorphic ecdysis. The animals depicted in each column are representative of the fifteen animals injected on that day.

What causes the JH induced molting delay in injection groups 2 through 4 of Fig. 11 is open to speculation at this point. It may correspond to a similar phenomenon observed in last instar larval *Manduca sexta* in which JH in hypothesized to inhibit the release by the brain of prothoracotrophic hormone (Nijhout and Williams, 1974). A similar ability of JH to inhibit the initiation of a molting cycle has been observed in another lepidopteran, *Spodoptera littoralini* (Cymborowski and Stolarz, 1979). From an evolutionary point of view, the mutual antagonisms of ecdysone on

the JH induced vitellogenic response and JH on the molting response may both be relics of the primitive regulatory system in the apterygotes, in which molting and reproduction alternate in a mutually exclusive way (Fig. la).

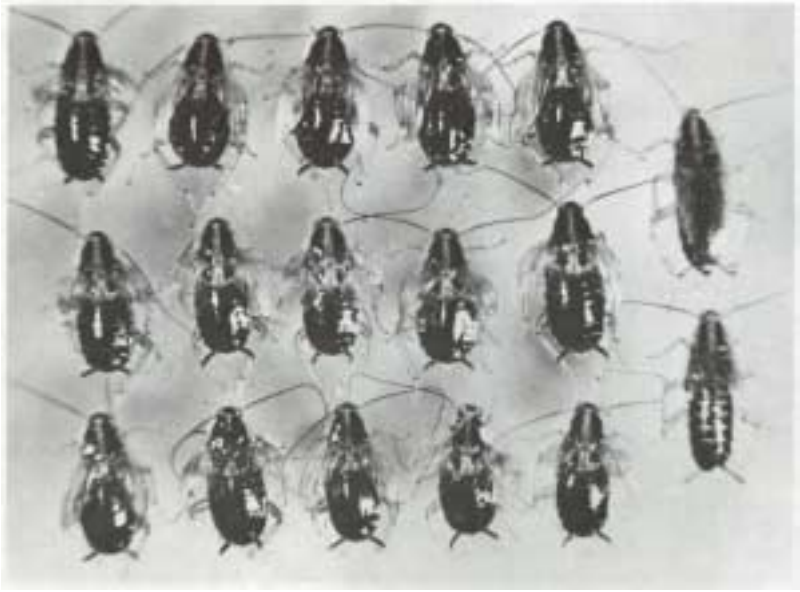

Fig.12 Juvenilized adult *Blattella germanica* resulting from nymphs injected with 0.5 µg of JH-I on day five after feeding. Two normal adult females, one with its wings clipped to bare its abdomen, are included for comparison at the right of the juvenilized adults.

Degrees of Metamorphosis at the Last Larval Instar

As mentioned above, even in highly inbred lines of *B. germanica* the instar of metamorphosis is not a determined feature. In one inbred strain (23 generations of brother-sister mating), under a particular set of culture conditions, 50% of the females are nymphs in the V instar and the remainder in the VI instar. All of these animals are close to isogenic yet when nymphal females of the two instars are challenged with a maximal dose of JH at various times in their stadium, they respond with different levels of Vg in their serum (Fig. 13). The two-fold difference in vitellogenic capacity of these genetically (perhaps) but superficially identical nymphs reflects some as yet unexplained relationship between instar and metamorphosis of the fat body. The two fold difference in secretion is not likely to be explained by the minor differences in blood volume or fat body mass and may represent another example of a stage in fat body metamorphosis.

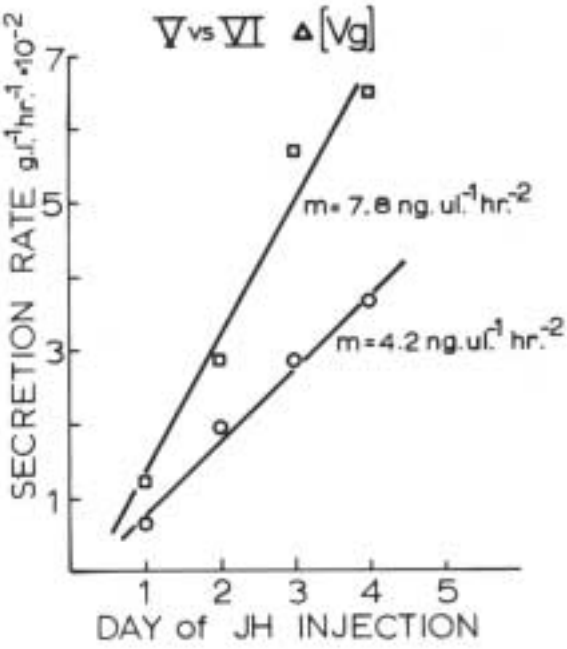

Fig.13 Instar dependent competence to secrete vitellogenin (Vg) in *Blattella germanica* nymphs. Each point represents the slope of a JH induced accumulation of Vg in the serum of a group of nymphs injected with JH on the day indicated on the ordinate. The V instar nymphs' secretion rates (circles) are at each time of the molting cycle about half the secretion rate of comparable VI instar nymphs (squares).

The maximum rate of secretion observed for the VI instar nymphs (650ng/µ1/hr, 4 days after feeding), approaches the rate observed in ovariectomized adult females (Fig. 6), 730 ng/µ1/hr. With respect to rate of secretion, the VI instar nymphal fat body is close to completely metamorphosed five days prior to the imaginal ecdysis. The V instar nymph, five days prior to its imaginal ecdysis, is only capable of secreting at half that rate. The corresponding adults from these two types of nymphs produce approximately the same weight of eggs, so the eventual capacity of the fat bodies of the two types of nymphs is not in question. This kind of investigation, which contrasts genetically uniform but developmentally different or experimentally altered animals, will, hopefully, provide a biochemical explanation for these developmental phenomena.

The above results also sound a cautionary note to anyone studying the metamorphosis of the fat body in hemimetabolous insects and particularly, cockroaches. In order to obtain consistent results one must know more than the animal's apparent developmental stage (i.e., penultimate larva or nymph); one must know the number of larval instars that have preceded the metamorphic instar. Efforts to predict the developmental stages of cockroaches using simple structure:size ratios are successful in identifying the early in-stars, but fail particularly with respect to distinguishing the critical penultimate larval stage and discriminating between V and VI instar nymphs (Tanaka and Hasegawa, 1979). The only alternative to the uncertainty is to employ some form of genetic canalization and synchronous culture technique (Kunkel, 1966; 1977), in which the developmental and chronological ages of the animals are known precisely.

PROSPECTS AND ALTERNATIVES

An argument for a minimal complexity model of metamorphosis has been presented suggesting that such a model would allow easier access to the mechanism of metamorphosis and avoid the confusion of the excessive number of differences between the larval and adult physiological phenotypes. At the moment it may seem far fetched to suggest that the advantages posed by the cockroach, *Blattella germanica,* or any other hemimetabolous insect for that matter, could make up for the deficit in experience with its biology compared with that of the holometabolous models of development, *Drosophila,* and the giant silk moths. Surely, the exquisitely developed genetics and emerging molecular biology of *Drosophila* makes that organism difficult to compete with.

However, a respectable genetics is developing for *B. germanica* (Ross and Cochran, 1975). Inbred strains of this species are available, as described above. This species in particular compares favorably with the laboratory mouse in generation time, ten weeks for the cockroach at 30° C versus nine weeks for the mouse. Combining the capacity to do genetics with the ability to synchronize the molting cycles of the cockroach results in an experimental animal well suited for studying the process of metamorphosis.

Two major gene products show dramatic changes during metamorphosis in *Blattella*: the LSP gene is shut off, and the Vg gene is turned on. This reciprocal change epitomizes the phenomenon of

metamorphosis. The fact that the Vg gene is actually first inducible in the pharate nymph by injections of exogenous JH, can be construed as support of the concept of a stepwise metamorphic process in the hemimetabola. However, it might also be evidence for independence of the two gross metamorphic products, i.e., reproductive competence, and flight associated cessation of molting. Metamorphosis may have evolved as a gradual accretion of independently controlled changes, each capturing JH titer as its signal for activation.

Clearly, we must learn more about the metamorphic process, and the modulation of Vg production is a significant activity which can be followed in detail. Two steps in the turning on of vitellogenic competence have been described, a difference in lag time between the larval and adult Vg responses to JH, and a difference in maximum inducible Vg secretion rate for different instar nymphs.

The difference in lag time between nymphs and adults has a number of possible explanations. The extra lag in the nymphal fat body response to JH may represent the time necessary to develop the enzymatic and ultrastructural machinery for processing the pre- and provitellogenin molecules. Currently we are working on characterizing the nonprotein portion of Vg, including its phosphate and oligosaccharide (Kunkel et al., 1978, and in preparation). The typical branched oligosaccharide of Vg seems to be a uniform 10 or 11 mannose residues long, attached through a chitobiosyl linkage to an asparagine residue on the protein backbone. The phosphate is attached relatively close to, but not on, the oligosaccharide. Presumably, specific glycosyl transferases and kinases would have to be in existence prior to the processing of Vg before its secretion into the hemolymph. These may need to be induced by a primary response to JH similar to that seen in nymphs.

The difference in maximal secretion rate by V versus VI instar nymphs could have two non trivial explanations: either each individual fat body cell gradually develops its competence to respond vitellogenically to JH and is present at different stages of its development in the above two types of nymphs, or fat body cells as a population are only gradually recruited to be able to respond to JH and the two types of nymphs represent recruitments of different proportions of the total population of fat body cells. Either of the alternatives would be interesting if confirmed.

When the mechanism of metamorphosis of the expression of the

Vg gene is worked out, it will become possible to ask whether the turning off of the larval gene for LSP is metamorphically tied to, or merely a concomitant of the gain of vitellogenic competence.

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